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ETIOLOGY OF YELLOW FEVER.

XII. Chemotherapy versus Serotherapy in Experimental Infection with Leptospira icteroides.

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(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, April 3, 1920.)

The work of Ehrlich and Hata[1] on the therapeutic effects of arsenical organic compounds in various infections caused by spirochetal or protozoan organisms has led to considerable investigation by later workers, notably Jacobs, Heidelberger, Amoss, and Bull[2–5] and Pearce and Brown,[6] of the extent to which this kind of curative method is applicable. The present experiments were undertaken, not as research in chemotherapy, but merely as part of a routine study of experimental infection in guinea pigs with *Leptospira icteroides*, derived from certain cases of yellow fever.[7] It was of particular interest to ascertain how *Leptospira icteroides* would behave toward the two widely employed chemotherapeutic agents, salvarsan and neosalvarsan, and what difference, if any, there is between its behavior and that of the inciting agent of infectious jaundice in this respect, for the latter has been extensively studied by Inada, Ido, and their collaborators,[8] as well as

[1] Ehrlich, P., and Hata, S., Die experimentelle Chemotherapie der Spirillosen, Berlin, 1910.

[2] Jacobs, W. A., *J. Exp. Med.*, 1916, xxiii, 563.

[3] Jacobs, W. A., Heidelberger, M., and Amoss, H. L., *J. Exp. Med.*, 1916, xxiii, 569.

[4] Jacobs, W. A., Heidelberger, M., and Bull, C. G., *J. Exp. Med.*, 1916, xxiii, 577.

[5] Jacobs, W. A., and Heidelberger, M., *J. Biol. Chem.*, 1915, xx, 513, 659, 685; xxi, 103, 145, 403, 439, 455, 465.

[6] Pearce, L., and Brown, W. H., *J. Exp. Med.*, 1918, xxviii, 109.

[7] Noguchi, H., *J. Exp. Med.*, 1919, xxix, 547, 565, 585; xxx, 1, 9, 13, 87, 95, 401; 1920, xxxi, 135, 159.

[8] Inada, R., Ido, Y., Hoki, R., Kaneko, R., and Ito, H., *J. Exp. Med.*, 1916, xxiii, 377. Kaneko, R., and Okuda, K., *Verhandl. japan. path. Ges.*, 1916, vi, 49.

by European investigators, especially Uhlenhuth and Fromme.[9] A large number of experiments with salvarsan and neosalvarsan, atoxyl, and arsacetin against *Leptospira icterohæmorrhagiæ* was also carried out in 1917, with negative results.[10] Being regarded by some as a spirochete, *Leptospira icterohæmorrhagiæ* was expected to yield to chemotherapy by means of salvarsan or its derivative, but Inada and Ido and others soon found that neither salvarsan nor neosalvarsan had any definite therapeutic value in infections with this organism. In this respect, at least, the organism discovered by Inada and Ido in infectious jaundice did not behave like the other pathogenic spirochetes, and this characteristic may be regarded as giving further support to the opinion that the leptospira group of organisms forms a type of its own and differs from the other pathogenic genera of Spirochætoidea. The experiments here reported concern the behavior of *Leptospira icteroides* toward the arsenical compounds, not only in the animal body, but also *in vitro*, and the effect upon the organism of salvarsanized serum. From the practical standpoint it seemed advisable to include also a brief comparison of the action of the arsenical preparations and that of anti-*icteroides* immune serum upon *Leptospira icteroides* in experimental infection and *in vitro*.

Effect of Salvarsan and Neosalvarsan upon the Course of Experimental Infection of Guinea Pigs Inoculated with Leptospira icteroides.

Several series of experiments were performed in order to ascertain whether administration of salvarsan or neosalvarsan simultaneously with the inoculation or shortly afterwards would in any way influence the development of the experimental leptospiral infection in guinea pigs. If the virulence of *Leptospira icteroides* for this animal were constant a few series of experiments only would have been sufficient to determine the point, but owing to the variable character of the pathogenicity of the organism for individual guinea pigs it was necessary to repeat similar experiments. In some series control animals survived or escaped infection, hence the interpretation of the effect of the drugs was rendered inconclusive. In the earlier experiments

[9] Uhlenhuth and Fromme, *Z. Immunitätsforsch., Orig.*, 1916, xxv, 418.

[10] Noguchi, H., unpublished results.

the amounts of infecting material were near a single lethal dose, even subminimum lethal doses being used; *i.e.*, a quantity capable of producing in the majority of instances a mild infection with recovery. The mode of experiment was practically the same in all the series. In one instance an unneutralized solution of salvarsan was employed, otherwise salvarsan has been used as an alkaline solution. The injection of the infecting material was intraperitoneal and that of the drugs subcutaneous.

TABLE I.

Inoculation of Infecting Material into Guinea Pigs Followed by Injection of Drug.

November 27, 1919. The amount of infecting material used was 0.5 cc.

Amount of arsaminol-natrium.	Course of disease.	Result.	Remarks.
gm.			
0.03	Mild; 6 to 8 days.	Recovered.	Died in 15 days; no jaundice.
0.02	Moderate (+); 6 to 11 days.	"	No jaundice.
0.01	Severe (++++); 6 to 11 days.	"	Died in 13 days; jaundice fading.
0.005	Mild; 7 to 9 days.	"	No jaundice.
0.0025	Severe (++++); 6 to 11 days.	"	Killed in 11 days; typical lesions.
0.001	Died in 6 days.	Typical.	
0.0005	" " 9 "	"	
Control.	Severe (+++); killed in 6 days.	"	
"	Moderate (++); 7 to 8 days.	Recovered.	
"	Mild (±); 7 days.	"	

Series 1.—November 27, 1919. The infecting material consisted of a mixture of citrate blood (showing the leptospiras) of a guinea pig experimentally infected with Guayaquil Strain 1 and a rich culture of the same strain. Ten guinea pigs were inoculated intraperitoneally, each with 0.5 cc. of the mixture, and all but three (controls) then received subcutaneously a solution of arsaminolnatrium (a Japanese preparation of neosalvarsan), the quantities given ranging from 0.03 to 0.0005 gm. (Table I).

The amount of infecting material used in this series was near a single minimum lethal dose, as shown in the control animals and also in those which received the smallest amounts of the drug. It is noteworthy that in the three guinea pigs which received 0.03, 0.02,

and 0.005 gm. of the drug, respectively, there was no jaundice at any period of the infection.

Series 2.—December 3, 1919. The infecting material consisted of a mixture of 1 cc. each of cultures of Guayaquil Strains 1 and 5, and 7 cc. of heart's blood from a guinea pig infected typically with Strain 1. 0.5 cc. of the mixture was inoculated intraperitoneally into each of sixteen guinea pigs. One group of eight animals was then inoculated subcutaneously with an unneutralized solution of salvarsan and the other group of eight with the solution of neosalvarsan in doses

TABLE II.

Inoculation of Infecting Material into Guinea Pigs Followed by Injection of Drug.

December 3, 1919. The amount of infecting material used for the treated animals was 0.5 cc.

Treated animals.			Untreated controls.	
Amount of drug.	Course of disease.		Test for virulence of infecting material used.	Course of disease.
	Salvarsan (acid solution).	Neosalvarsan.		
gm.			*cc.*	
0.03	Mild; recovery.	Mild; recovery.	1.0	Severe (++++); recovery.
0.02	No symptoms.	Severe (+++); recovery.	0.5	Mild; recovery.
			0.2	Moderate (++); recovery.
0.01	" "	Mild; recovery.		
0.005	" "	No symptoms.	0.1	No symptoms.
0.002	Died in 12 days; typical.	" "		
0.001	No symptoms.	" "		
0.0005	" "	Died in 13 days; typical.		
0.0002	" "	Severe (+++); recovery.		

varying from 0.03 to 0.0002 gm. There were four control guinea pigs which received varying doses of the infecting material and were not treated with the drug (Table II).

The results of these experiments suggest a possible slight protective effect of the drugs in some individuals, although, from the mildness of the infection in the control animals the survival of those individuals may also be explained on the basis of a natural resistance to *Lepto-*

spira icteroides. It is of interest to note that more guinea pigs among those treated with salvarsan escaped the infection than among those treated with neosalvarsan. The indecisive character of the experiments made necessary another series with multiple minimum lethal doses. In Series 3 at least 50 minimum lethal doses were used for each guinea pig.

Series 3.—March 13, 1920. The infecting material consisted of a mixture of 5 cc. of a culture of Guayaquil Strain 1, 5 cc. of kidney emulsion, and 5 cc. of citrated heart's blood from a guinea pig inoculated with the same strain 4 days previously. In order to produce a fatal infection in guinea pigs 0.5 cc. of the mixture was inoculated intraperitoneally into each animal. As the protocol shows, 0.01 cc. of this material killed a control animal in 7 days with typical symptoms, and a much smaller quantity would have been sufficient to cause a fatal infection, although the minimum lethal dose was not determined in the present series of experiments.

Salvarsan was dissolved in sterile distilled water and a 0.1 N sodium hydroxide solution was gradually added until the precipitate first formed was completely redissolved. The final concentration of the drug was made 0.1 gm. per 10 cc. (stock solution). Neosalvarsan was dissolved in sterile distilled water in the ratio of 0.1 gm. per 10 cc. (stock solution). Further dilutions were made with 0.9 per cent salt solution.

The animals were inoculated with 0.5 cc. of the infecting material intraperitoneally, and within about 30 minutes varying amounts of salvarsan and neosalvarsan solution were injected subcutaneously. The amounts of the drug were 0.00005, 0.0001, 0.0002, 0.0005, 0.001, 0.002, and 0.003 gm. for guinea pigs of 350 gm., and each dose was contained in from 0.5 to 3 cc. of fluid, according to convenience in measurement. Table III gives the results.

Series 4.—March 18, 1920. Although it was still too early to know the results of the series of March 13, another series was begun on March 18 in which larger doses (0.01, 0.02, and 0.03 gm.) of salvarsan and neosalvarsan were used. The solutions of the drugs were freshly prepared from another set of ampules, and the infecting material consisted of a guinea pig kidney emulsion of Guayaquil Strain 1 of *Leptospira icteroides*. Other technical details were the same as in the previous experiments. Table IV gives the results.

The experiments of Series 3 (March 13) show that guinea pigs receiving at least 50 minimum lethal doses of a strain of *Leptospira icteroides* intraperitoneally and varying quantities of salvarsan or neosalvarsan subcutaneously within 30 minutes from the time of inoculation of *icteroides* undergo a typical course of leptospira infection, resulting in the majority of instances in death within a

period the variations of which may be considered usual in such a series of experiments. With salvarsan there were two instances of recovery after a severe infection coinciding with elevated doses of the drug (0.001 and 0.003 gm.), while in animals receiving less of the

TABLE III.

Inoculation of Infecting Material into Guinea Pigs Followed by Injection of Drug.

March 13, 1920. The amount of infecting material used for the treated animals was 0.5 cc.

Treated animals.			Untreated controls.	
Amount of drug.	Course of disease.		Test for virulence of infecting material used.	Course of disease.
	Salvarsan.	Neosalvarsan.		
gm.			*cc.*	
0.00005	Died in 5 days; typical.	Recovery after a mild infection, with definite jaundice from Mar. 20–24, 1920.	0.5	Died in 6 days; typical.
			0.1	Died in 7 days; typical.
0.0001	" " 8 " "	Died in 5 days; typical.	0.01	Died in 7 days; typical.
0.0002	" " 5 " "	" " 6 " "		
0.0005	" " 6 " "	" " 7 " "		
0.001	Recovery after a typical infection, with intense jaundice from Mar. 19–25, 1920.	" " 9 " "		
0.002	Died in 6 days; typical.	Recovery after a mild infection, with jaundice from Mar. 19–23, 1920.		
0.003	Recovery after a typical infection lasting from Mar. 18–20, 1920.	Recovery after a severe infection, with intense jaundice from Mar. 18–23, 1920.		

drug death occurred in two instances 2 days earlier than in controls, a fact worthy of notice. With neosalvarsan the number of recovered animals was three, two with the largest doses of the drug (0.002 and 0.003 gm.) and one with the minutest dose employed (0.00005 gm.).

All the rest died in from 5 to 9 days with typical symptoms. The results both with salvarsan and with neosalvarsan suggest that these arsenical compounds, when employed in certain quantities, may somewhat modify the severity of the infection and occasionally save a guinea pig from death, although failing to suppress the infection completely.

The results of the experiments of Series 4 (March 18) were less favorable than those of Series 3. Here, of the two controls one died in 12 days and the other recovered after a definite infection. Two

TABLE IV.

Inoculation of Infecting Material into Guinea Pigs Followed by Injection of Drug.

March 18, 1920. The amount of infecting material used for the treated animals was 0.5 cc.

Treated animals.			Untreated controls.	
Amount of drug.	Course of disease.		Test for virulence of infecting material used.	Course of disease.
	Salvarsan.	Neosalvarsan.		
gm.			*cc.*	
0.01	Died in 11 days; typical.	Died in 12 days; typical.	0.5	Died in 12 days; typical.
0.02	Had fever but no jaundice; recovery.	" " 12 " "	0.5	Recovery after a definite infection, with intense jaundice from Mar. 30–31, 1920.
0.03	Died in 11 days; typical.	Had fever but no jaundice; recovery.		

of the three guinea pigs treated, either with salvarsan or neosalvarsan, died in from 11 to 12 days, and the one animal which survived in each set had fever indicative of an abortive leptospira infection.

From the therapeutic standpoint neither salvarsan nor neosalvarsan is of any value in experimental infections of guinea pigs with *Leptospira icteroides*. In contrast, it may be interesting to include here a protocol illustrating the highly specific protective value of an anti-*icteroides* serum (horse). The amount of the immune serum required to protect a guinea pig from an infection with *Leptospira icteroides* is exceedingly minute.

Contrasted Effect of Anti-icteroides Immune Serum in Vivo.

March 11, 1920. The experiments with the immune serum were undertaken with the same strain of *Leptospira icteroides* (Guayaquil No. 1) that was used in the chemotherapeutic experiments already described. The material was a mixture of the emulsions of kidney and liver from a guinea pig which was showing early symptoms of the leptospira infection. As the protocol shows, the minimum lethal dose of this emulsion was such that 1 cc. of a 1:10,000 dilution of it killed a guinea pig with typical symptoms in 12 days, and the same quantity of a 1:1,000 or 1:100 in 10 days. 0.5 cc. of the original emulsion killed a guinea pig in 8 days.

TABLE V.

Inoculation of Infecting Material into Guinea Pigs Followed by Injection of Anti-icteroides Serum.

March 11, 1920. The amount of infecting material used for the treated animals was 0.5 cc.

Treated animals.			Untreated controls.	
Anti-*icteroides* immune serum.		Course of disease.	Test for virulence of infecting material used.	Course of disease.
Amount.	Dilution.			
cc.			*cc.*	
1	1:100,000	Recovery after a mild infection.	0.5	Died in 8 days; typical.
			0.1	Recovery after a severe infection (exceptional resistance).
1	1:10,000	No symptoms.		
1	1:1,000	" " (suspicion of trace of jaundice on Mar. 20, which had disappeared the following morning).	0.01	Died in 10 days; typical
			0.001	" " 10 " "
			0.0001	" " 12 " "
			0.00001	No symptoms.
1	1:100	No symptoms.	0.000001	" "
1	1:10	" "		

For infecting guinea pigs to be treated with anti-*icteroides* serum, 0.5 cc. (about 5,000 minimum lethal doses) of the same emulsion was intraperitoneally injected. The different amounts of the anti-*icteroides* serum (collected from Horse 2 on February 25, 1920) were then injected, also intraperitoneally, within half an hour after the inoculation. The amounts of immune serum were 0.00001, 0.0001, 0.001, 0.01, and 0.1 cc. Table V is a record of the results.

According to a conservative estimate, therefore, the power of the immune serum is such that 1 cc. of a 1:10,000 dilution prevented an infection when the dose of the infecting material was 5,000 minimum

lethal doses. In other words, 1 cc. of the serum had the power to protect a guinea pig of 350 gm. body weight against 50,000,000 (10,000 × 5,000) minimum lethal doses when administered intraperitoneally 30 minutes after intraperitoneal inoculation. The specific protective property of the immune serum is indisputably highly efficacious as compared with salvarsan or neosalvarsan, the value of which is at least doubtful. A point of considerable importance is that in certain guinea pigs receiving small quantities of salvarsan and neosalvarsan the period before death seemed to be shortened by 2 days (5 days), as compared with the average period in untreated control animals (7 days). It may be that the predilection of arsenic compounds for the renal tissues had a definite predisposing effect, due to chemical injury, upon this organ, which *Leptospira icteroides* also attacks particularly.

Direct Action of Salvarsan and Neosalvarsan upon Leptospira icteroides.

Ehrlich makes a special point of having found, in his quest for chemotherapeutic agents, that a preparation whose destructive power on a microbic organism is great *in vitro* may have no, or only a slight antagonistic effect when introduced into the animal body, or the relation between the effect manifested *in vitro* and *in vivo* may be the reverse. Ehrlich's efforts to find a chemotherapeutic preparation were principally directed toward its effect *in vivo*, since the pathogenic parasites with which he was working belonged to the class of protozoa or a class closely allied to it, and there were no virulent cultures on hand to be tested *in vitro* as well as *in vivo*. Certain arsenic compounds elaborated by Ehrlich and his coworkers displayed a highly sterilizing effect on various spirochetoid organisms when introduced into the animal body infected with them, while the direct effect of these preparations upon the same organisms *in vitro* was almost nil. Ehrlich interpreted the highly parasiticidal properties of these compounds as due to a certain modification (reduction) in the animal body of substances otherwise comparatively inert. Salvarsan and *Treponema pallidum* constitute a good example in point.

Leptospira icteroides is resistant to saponin,[11] a property which alone would serve to differentiate it from certain other groups of spiro-

[11] Noguchi, H., *J. Exp. Med.*, 1919, xxx, 13.

chetoid organisms (*Treponema* and *Spironema*). The fact that *in vivo* the leptospiras were scarcely influenced by the introduction of salvarsan or neosalvarsan constituted another point of dissimilarity between the leptospira and other spirochetal parasites. Hence the effect of salvarsan or neosalvarsan upon the leptospira from yellow fever cases *in vitro* presented a further interesting subject for study. Several experiments were also performed to determine the behavior of blood serum derived from a rabbit which had been injected intravenously 1 hour previously with salvarsan or neosalvarsan in considerable quantities; that is, a study was made of the effect of salvarsanized serum, as well as of drug solutions, upon *icteroides in vitro*.

TABLE VI.

Addition of Drug to Culture of Leptospira icteroides.

November 26, 1919.

Final concentration of arsaminolnatrium.*	Result.
1:2,000	All inactive and most have disappeared.
1:6,000	" " " some degenerated.
1:20,000	" "
1:60,000	" " thinner, and smoother.
1:200,000	" "
1:600,000	For the most part inactive.
1:2,000,000	" " " " " some still active.
Water (control).	" " " " active, a few inactive.

* The concentration resulting from the mixture of the drug solutions with the culture is given.

To each of several tubes containing 1 cc. of a culture of Guayaquil Strain 6 was added 1 cc. of different dilutions of salvarsan, neosalvarsan, or arsaminolnatrium. The mixtures were allowed to stand at room temperature and examined after 2 or 2½ hours, and again after 24 or 48 hours. As Tables VI and VII show, sufficient quantities of the drugs rendered the leptospiras immotile, and brought about general disintegration. The highest dilution of any of the drugs which still killed the organism was about 1:200,000.

In testing the leptospiricidal strength of the solutions of salvarsan or neosalvarsan, it was important to take note of the reactions of the solutions. Salvarsan, according to the usual practice, is first treated with sodium hydroxide solution until completely precipitated out

at the point of neutral reaction. In order to obtain a clear solution it is necessary to add more alkali until the precipitate completely dissolves. At this point the solution is no longer slightly, but intensely alkaline. By reducing the alkalinity with hydrochloric acid to a reaction of about pH 8 a bulky precipitate is once more formed, and at pH 7 the entire substance flocculates out of the solution. In making a microbicidal titration a clear alkaline solution was used in ascending dilutions to eliminate the destructive effect of the reaction alone. It was found that the 1:1,000 dilution was still too strongly

TABLE VII.

Addition of Drugs to Culture of Leptospira icteroides.

December 3, 1919.

Final concentration of the drug.	Salvarsan.		Neosalvarsan.	
	2½ hrs.	24 hrs.	2½ hrs.	24 hrs.
1:200	Precipitate (?).	Precipitate heavy.	All inactive.	All immobilized.
1:2,000	Some active; precipitate.	All immobile; precipitate.	" "	" "
1:20,000	Some active; precipitate.	All immobile; precipitate.	" "	" "
1:200,000	For the most part active.	All immobile.	" "	" "
1:2,000,000	For the most part active.	For the most part immobile; few active.	For the most part active.	Few active.
Saline (control).	All active.	All active.	All active.	For the most part active.

alkaline to be used (far beyond pH 8), the leptospiras dying rapidly in such a solution, while dilutions higher than 1:10,000 had a reaction which was practically that of the diluent (saline solution), pH 7. Neosalvarsan dissolved in distilled water in 1:100 dilution is a clear amber-yellow and shows to phenol red an intense red color; in diluting to 1:1,000 with saline solution its reaction approaches pH 7.2; in 1:10,000 dilution it is no longer perceptible to phenol red. In actual experiments, however, 1 cc. of each of the dilutions of the drugs was mixed with 1 cc. of a rich culture of one of the *icteroides* strains which

had a reaction of pH 7.4. This mixing brought down the pH value of the lower dilutions and raised that of the higher. The reactions between pH 7 and pH 7.8 are well borne by *icteroides*, the optimum being near pH 7.2 to 7.4. A reaction beyond pH 8 or below pH 6.6 is unsuitable for the existence of the organism. The devitalizing action of salvarsan and neosalvarsan, even in optimum reactions, is rather slow, a contact of many hours being required before death ensues, as the protocol shows.

TABLE VIII.

Addition of Drugs to Culture of Leptospira icteroides.

March 18, 1920.

Final concentration of the drug.	Salvarsan.			Neosalvarsan.		
	Reaction.	After 15 min.	After 18 hrs.	Reaction.	After 15 min.	After 18 hrs.
1:200	Intensely alkaline.	Dead.		Slightly over pH 8.	Active.	Dead.
1:2,000	Intensely alkaline.	"		pH 7.2	"	"
1:20,000	pH 7.2	Active.	Dead.	pH 7.2	"	"
1:200,000	pH 7.2	"	"	pH 7.2	"	"
1:2,000,000	pH 7.2	"	" (?).	pH 7.2	"	Active.*
Saline control (no drug).	pH 7.2	"	Active.	pH 7.2	"	"

* All found dead after 96 hours; the control tube was lost through contamination.

To each of several tubes containing 1 cc. of the same culture of *icteroides*, Guayaquil Strain 5 (pH 7.4), was added 1 cc. of several different dilutions of drugs, 1:100, 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000 of alkalinized salvarsan solution and neosalvarsan. The mixtures were allowed to stand at 26°C. and the contents examined under the dark-field microscope after 15 minutes and again after 18 hours. The results are recorded in Table VIII.

It is evident that *Leptospira icteroides* is highly sensitive to the action of salvarsan and neosalvarsan, but their action is comparatively slow, requiring many hours contact. The effects of the drugs in a culture medium are found to be approximately the same as in the case of direct mixing of culture and solutions.

Various dilutions of the drugs were added to the usual medium (rabbit serum, 25 per cent, agar, 0.3 per cent, total volume, 6 cc.). The culture used was Guayaquil Strain 1, and the tubes were allowed to stand for 5 days at room temperature (26°C.). A medium containing salvarsan or neosalvarsan in a ratio of more than 1:200,000 was found to be unsuitable for the growth of the organism (Table IX). No attempt was made to determine quantitatively the exact leptospiricidal titers of the two drugs.

TABLE IX.

*Addition of Drugs to Culture Media.**

March 6, 1920.

Final concentration of the drug.	Salvarsan.	Neosalvarsan.
1:600	−	−
1:1,800	−	−
1:6,000	−	−
1:18,000	−	−
1:60,000	−	−
1:180,000	−	−
1:600,000	+	<+
1:1,800,000	+	+
1:6,000,000	+	+
Control.	+	+
"	+	+

* The medium consisted of rabbit serum, 25 per cent, agar, 0.3 per cent, total volume, 6 cc.

The changes in the color of salvarsan, when observed after 12 days standing, were noticeable in the tubes containing dilutions from 1:100,000 up, while with neosalvarsan the changes were not found in dilutions below 1:10,000.

Effect of Salvarsanized and Neosalvarsanized Serum.

The problem here was to determine the effect of the living body upon the drugs when the latter were introduced into the blood circulation. It has been assumed that in the animal or human body they are converted into highly spirocheticidal substances, as is said to be the case with respect to the organisms of syphilis, yaws, and relapsing fevers. It has already been shown that these drugs are practically without effect *in vivo* upon the course of the *icteroides* infection, and that they are highly destructive to the organism *in vitro*. It was this discrepancy which suggested the study of the salvarsanized serum.

The mode of experiment was to inject a rabbit intravenously with salvarsan or neosalvarsan in a ratio of 0.05 gm. per kilo of body weight, the blood being drawn 1 hour after the time of injection. Two rabbits, weighing 1,500 and 2,000 gm. respectively, were injected on March 18, 1920, intravenously, one with salvarsan (alkaline), the other with neosalvarsan. After 1 hour they were killed for the blood. The clear serums were collected the next day and used in the active state in order to determine whether they were in any way different from a normal rabbit serum when mixed *in vitro* with the same quantity (1 cc. in each case) of a rich culture of Guayaquil Strain 5. The mixtures were kept in a thermostat at 28°C. during the period of observation. Table X gives the results.

The experiments demonstrated clearly the difference between the normal and the salvarsanized rabbit serums. All the leptospiras mixed with the latter died within 72 hours, while those in normal rabbit

TABLE X.

Effect of Salvarsanized, Neosalvarsanized, and Normal Rabbit Serum in Vitro.

March 18, 1920.

Duration of contact.	Salvarsanized rabbit serum, 1 cc., plus culture, 1 cc.	Neosalvarsanized rabbit serum, 1 cc., plus culture, 1 cc.	Normal rabbit serum, 1 cc., plus culture, 1 cc.
hrs.			
1	All active.	All active.	All active.
18	For the most part motile, but sluggish.	For the most part sluggish.	" "
48	No observation.	No observation.	No observation.
72	All dead and degenerated.	All dead and degenerated.	All active and multiplying.

serum steadily multiplied. It may be mentioned that the reactions of the drugged serums as well as the reaction of the normal serum were pH 7.4, hence the question of hydrogen ion concentration does not enter into the present comparative study. At the end of 18 hours the organisms were already less active in the drugged serums than in the normal serum.

It occurred to me that the cause of this slow leptospiricidal action of the salvarsanized and neosalvarsanized serums might be due to a gradual development of injurious substances by slow oxidation of the drugs. If this were the case we should find in these tubes a powerful and rapidly acting leptospiricidal substance at the end of 72 hours of exposure to the same experimental conditions. Two different experiments were carried out to ascertain this point.

In the first experiment 0.5 cc. of a rich culture of the Merida strain[12] of *icteroides* was added to each of the three tubes containing salvarsanized, neosalvarsanized, and normal serum respectively. The results were similar to those observed with the Strain 5 culture; that is, there was no effect upon the organisms during the 1st hour; they were all active at the end of that time. After 24 hours the leptospiras in the salvarsanized serum were all dead, but there were many active survivors in the neosalvarsanized serum. After 48 hours, however, they were for the most part dead in the neosalvarsanized serum, but all were active in the tube containing the normal serum. In the second experiment three tubes containing 1 cc. of each of the serums were placed at 28°C. for 72 hours, then 1 cc. of the rich Merida culture was added to each. The mixtures were again put at 28°C. for the period of observation. Table XI gives the results of this experiment.

TABLE XI.

*Effect on Leptospira icteroides (Merida Culture) of Salvarsanized, Neosalvarsanized, and Normal Rabbit Serums after the Serums Had Stood for 72 Hours in the Incubator at 28°C.**

Experiment of Mar. 22, 1920.	Salvarsanized serum, 1 cc., plus culture, 1 cc.	Neosalvarsanized serum, 1 cc., plus culture, 1 cc.	Normal serum, 1 cc., plus culture, 1 cc.
After 1 hr.	All active.	All active.	All active.
" 18 hrs.	Some active.	Many active.	" "
" 48 "	All dead.	All dead.	" "

* There was a slight shift of pH value (to pH 7.8) in these serums on standing, but they were brought back to pH 7.6 by the addition of the culture.

These two series of experiments indicate that a certain antagonistic substance seems to have developed in the tubes containing the salvarsanized and neosalvarsanized serums during the period of 72 hours at 28°C., as shown by an earlier death and degeneration of the leptospiras than was the case with the fresh samples of these serums. The difference was especially definite in the effects observed at the end of 18 hours with the first series. On the other hand, in no instance was there any rapid immobilization or destruction of the organisms, the drugs having exerted no perceptible effect upon them even after an hour's contact. At all events, no rapidly leptospiricidal substances could be demonstrated in the salvarsanized or neosalvarsanized rabbit serum after exposure to the air for 72 hours at 28°C. The death of the leptospiras in these drugged serums was slow but certain.

[12] This strain was isolated from a case of yellow fever in Merida, Mexico, and will be described in a later paper (Noguchi, H., and Kligler, I. J., *J. Exp. Med.*, 1920, xxxii, in press).

The question arises as to the form in which salvarsan or neosalvarsan existed in the blood serum of these two rabbits. This we do not know, but whatever its state, its ultimate concentration must correspond at most with a dilution of 1:20,000; that is, on the assumption that 0.05 gm. of the compound had diffused out in a space of 1,000 cc. In reality, the volume representing 1 kilo of body weight of the rabbit must be considerably smaller than 1,000 cc., hence the concentration of the drugs in the serum must have been stronger than 1:20,000. Other experiments (Table VII) showed that salvarsan or neosalvarsan added directly to a rich culture kills the latter within 24 hours; that is, in less time than that required with the salvarsanized or neosalvarsanized serum, which was at least 48 hours. Perhaps the arsenic compounds had undergone a modification in the animal body which converted them into substances operating much more slowly. As the animals did not urinate after the injection of the drugs up to the time of collecting the serum the slowness of action cannot be explained by elimination of the drug through the urine. Moreover, a dilution of 1:200,000 of the drugs when added directly to a culture caused the death of the latter in 18 hours. To summarize, then, serum drawn from rabbits at the end of 1 hour from the time of an intravenous injection of salvarsan or neosalvarsan in a ratio of 0.05 gm. per kilo of body weight possesses a slowly acting leptospiricidal property, which does not seem to be much increased in respect to rate of action by an exposure to the air for a period of 72 hours at 28°C.

Contrasted Effect of Anti-icteroides Immune Serum in Vitro.

Comparison has already been made of the chemotherapeutic value of salvarsan and neosalvarsan and the serotherapeutic value of anti-*icteroides* immune horse serum, showing the comparative inefficacy of these drugs and the highly potent specific protective property of the serum. Since the leptospiricidal power of the drugs was considerable *in vitro*, in sharp contrast with their lack of perceptible protective action *in vivo*, a similar comparison of the action *in vitro* of the immune serum and that of the drugs was of practical interest.

The same immune serum which was used in the experiments recorded in Table V was mixed with 1 cc. of a rich culture of Guayaquil Strain 5, the object being

to determine how high a dilution of the serum still had leptospiricidal power in the test-tube. The maximum dilution which caused complete immobilization and subsequent degeneration within 1 hour was 1:20, while a 1:200 dilution caused considerable but incomplete agglutination and degeneration within 18 hours. No effect whatever was perceptible in a mixture containing the serum in a dilution of 1:1,000 or less. The results of the experiment are recorded in Table XII.

When we place side by side this low titer *in vitro* of the immune serum (1 cc. of a 1:100 dilution to 1 cc. of culture) and its protective titer *in vivo* (1 cc. of a 1:10,000 dilution to 0.5 cc. of culture, or 5,000 minimum lethal doses), it is easy to conceive at once how completely

TABLE XII.

Effect of Anti-icteroides Immune Serum upon Leptospira icteroides in Vitro.

March 19, 1920.

Anti-*icteroides* immune serum.		After 1 hr.	After 18 hrs.
Amount.	Dilution.		
cc.			
1	1:10 (final dilution 1:20).	All dead.	All degenerated.
1	1:100 (" " 1:200).	For the most part active.	For the most part agglutinated and degenerated, but some still motile.
1	1:1,000 (" " 1:2,000).	All active.	All active.
1	1:10,000 (" " 1:20,000).	" "	" "
1	1:100,000 (" " 1:200,000).	" "	" "
1	Saline control.	" "	" "

reverse is the relation that exists between the behavior of the immune serum on the one hand and that of salvarsan and neosalvarsan on the other toward *Leptospira icteroides in vitro* and *in vivo*.

SUMMARY AND CONCLUSIONS.

In several series of experiments guinea pigs were variously infected with different amounts of *Leptospira icteroides*, either in the form of culture, organ emulsion from infected guinea pigs, or a mixture of both. The infecting materials were of different grades of virulence; in some series the amount given was near a single lethal dose, in others a subminimum lethal dose was given, *i.e.* causing mild infection

with recovery in the majority of animals, and in still others the animals were injected with at least 50 minimum lethal doses of a mixture of a culture and a highly virulent organ emulsion from a guinea pig. The animals were inoculated intraperitoneally, and within about 30 minutes each was injected subcutaneously with a different amount of salvarsan or neosalvarsan. The amounts injected were in most series 0.0005, 0.001, 0.002, 0.005, 0.01, 0.02, and 0.03 gm. per 350 to 450 gm. of body weight, and in one series, in addition to this dosage, 0.00005, 0.0001, and 0.0002 gm. were also tried.

Among the guinea pigs treated either with salvarsan or with neosalvarsan there were more recoveries than among the controls, but they were not in strict proportion to the amounts of the drugs injected. In the experiments with 50 minimum lethal doses of the infecting material there were several recoveries among those which received 0.001 to 0.002 to 0.003 gm., but all passed through a typical infection with all its symptoms. It is extremely doubtful, therefore, whether salvarsan or neosalvarsan mitigated the severity of the infection. The fact is noteworthy that in the same series of experiments the guinea pigs receiving 0.00005 and 0.0001 gm., or thereabout, of salvarsan died 1 to 2 days sooner than the controls, which died in 6 to 7 days. This suggests a possible earlier injury of the kidneys by the drugs, giving the leptospiras an easier and earlier access to, and localization in this organ. The inefficacy or dubious therapeutic value of salvarsan and neosalvarsan against the experimental *icteroides* infection of guinea pigs presents a close analogy to the observations already made by several investigators with *Leptospira icterohæmorrhagiæ*.

Several series of test-tube experiments were also made to determine the direct effect of salvarsan and neosalvarsan on *Leptospira icteroides* cultures. It was found, the injurious effect of alkalinity being eliminated, that the leptospiras remain motile for at least 1 hour in a concentration weaker than 1:10,000 of salvarsan or 1:1,000 of neosalvarsan. But they become gradually sluggish and succumb to the effect of the drugs at the end of 18 to 24 hours. The highest dilution which killed the leptospira in 18 hours was somewhere near 1:200,000.

When added to a culture medium, salvarsan and neosalvarsan both suppressed the growth of *icteroides* when their concentration in the

medium was 1:200,000. Hence these two drugs are highly poisonous for *Leptospira icteroides*.

The serums derived from rabbits which received 0.05 gm. of salvarsan or neosalvarsan per kilo of body weight 1 hour before bleeding proved to be very different from a normal rabbit serum in their behavior toward *Leptospira icteroides*. In the salvarsanized or neosalvarsanized serums the leptospiras remained active for at least 1 hour but appeared somewhat sluggish at the end of 18 hours, and were all dead and degenerated when examined after 48 hours. On the other hand, the leptospiras mixed with normal rabbit serum lived well and multiplied during the same period of time and under otherwise identical conditions (at 28°C.) To these tubes another portion of culture was added to determine whether or not a rapidly detrimental toxic substance had appeared in the drugged serum while standing for 72 hours, but the organisms remained still active at the end of 1 hour, 24 hours being required to kill them. In another experiment the salvarsanized and neosalvarsanized serums, together with normal serum as a control, were first left standing for 72 hours, after which period a rich culture of *icteroides* was introduced. The organisms remained uninfluenced for 1 hour in all the serums, but at the end of 24 hours many of those in the drugged serums were dead, and none was left alive at the end of 48 hours. In normal serum they steadily increased in numbers and were all active.

It is evident, then, that salvarsan or neosalvarsan introduced intravenously into the body of the rabbit is present in some form in the blood serum drawn at the end of 1 hour. The substance present in such serum has a slowly operating injurious effect upon *Leptospira icteroides*. The action of the drugs seems to be slower after passage through the animal body than before. If this phenomenon were to take place also in the infected body injected with these drugs, it is obvious that in a rapidly evolving infectious disease like yellow fever the progress of the infection will be too rapid to allow the drugs to exert their beneficial effect upon the course of the disease.

In direct contrast to the behavior of salvarsan and neosalvarsan *in vivo* and *in vitro*, anti-*icteroides* immune horse serum in a dose of 0.0001 cc., or 1 cc. of a 1:10,000 dilution, protected guinea pigs from an infection with at least 5,000 minimum lethal doses of *icteroides*

when injected simultaneously, but the same serum failed to exert any injurious effect upon the organism when mixed *in vitro* in a concentration weaker than 1:2,000. A rapid disintegration resulted with a concentration of 1:20 and almost complete agglutination and degeneration in 1:200.

The contrast between chemotherapy, as carried out with salvarsan and neosalvarsan, and serotherapy demonstrated with an immune serum is apparently of considerable practical significance.

STUDIES ON EXPERIMENTAL PNEUMONIA.

VIII. Experimental Streptococcus hæmolyticus Pneumonia in Monkeys.

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Plates 35 to 44.

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It has been shown in preceding papers[1,2] that pneumonia may be produced readily in normal monkeys by the intratracheal injection of pneumococcus and that the disease so produced is essentially identical in its clinical course, complications, and pathology with pneumococcus lobar pneumonia in man. In this paper experiments dealing with the production of pneumonia in monkeys with *Streptococcus hæmolyticus* are presented. These experiments were undertaken to determine whether hemolytic streptococcus pneumonia as it occurs in man could be reproduced experimentally in animals and for comparison with pneumococcus lobar pneumonia in monkeys. In addition the pathogenesis of hemolytic streptococcus pneumonia has been studied.

Method.

Monkeys were inoculated by direct intratracheal injection of broth cultures of *Streptococcus hæmolyticus*, and observations on the disease produced were made by the methods described in a preceding paper[1] on the production of pneumococcus pneumonia in monkeys. The strain of *Streptococcus hæmolyticus*[3] employed was originally isolated at autopsy from the lungs of a case of bronchopneumonia at Fort Sam Houston, Texas, in February, 1918, and possessed the typical

[1] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 403.

[2] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 445.

[3] We are indebted to Dr. A. R. Dochez for the strain of *Streptococcus hæmolyticus* used.

characteristics of this group of streptococci. It was a strongly Gram-positive coccus growing in chains of moderate length, bile-insoluble, and actively hemolytic. On the surface of rabbit blood agar plates it showed a wide zone of hemolysis, and when an 18 hour broth culture was mixed in equal quantity with a 5 per cent salt solution suspension of washed rabbit blood cells hemolysis was complete in 2 hours. It fermented lactose and salicin and failed to ferment mannitol, inulin, and raffinose. Its virulence had been raised by repeated mouse passage until 0.00000001 cc. of broth culture was lethal for a white mouse in 24 hours. Since this strain of *Streptococcus hæmolyticus* proved much less virulent for normal monkeys than the strains of pneumococcus employed in former experiments, the amount of culture injected varied from 0.1 to 10 cc. of an 18 hour plain broth culture. When larger amounts than 1 cc. were used the organisms were thrown down by centrifugalization and resuspended in portions of the supernatant broth so that the required amount of culture was contained in 1 cc. of medium, thus maintaining a constant amount of fluid injected into the trachea. Except in three instances, as indicated in the text, normal monkeys were used.

EXPERIMENTAL.

Production of Interstitial and Confluent Lobular Pneumonia by the Intratracheal Injection of Streptococcus hæmolyticus.

Nine normal monkeys were injected intratracheally with *Streptococcus hæmolyticus* with the successful production of varying degrees of interstitial or confluent lobular pneumonia in all the animals. The pneumonia was complicated in three cases by an extensive serofibrinous pleurisy, in one by pulmonary abscesses. It will be seen from Table I, in which the data of the experiments are summarized, that the disease was relatively mild. Death occurred in only three instances, in all of which a large amount of streptococcus was injected. Such a result is not surprising when it is recalled that *Streptococcus hæmolyticus* rarely attains sufficient virulence to produce a primary pneumonia in man, the pneumonia being nearly always secondary to measles, influenza, or some other preceding respiratory infection.

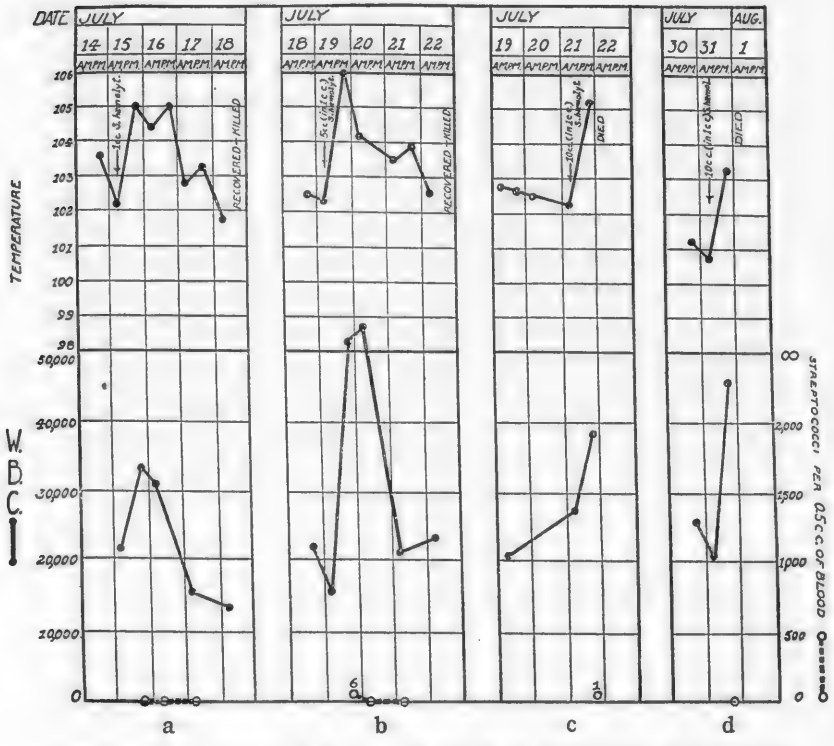
TABLE I.

Effect of Intratracheal Injection of Monkeys (Macacus syrichtus) with Streptococcus hæmolyticus.

Monkey No.	Weight.	Date of injection.	Amount of culture.	Result.	Autopsy.
	gm.	*1919*	*cc.*		
120	2,800	July 15	1.0	Pneumonia. Recovered on 4th day. Killed.	Interstitial pneumonia, L.L.; acute fibrinous pleuritis, left; chronic fibrous pleuritis, left.
121	3,150	" 15	1.0	Pneumonia. Recovered on 3rd day. Killed.	Interstitial and lobular pneumonia, R.L., R.M.
119	3,180	" 19	5.0 (in 1.0)	Pneumonia. Recovered on 4th day. Killed.	Interstitial and lobular pneumonia, R.L., R.U.
126	1,965	" 21	10.0 (" 1.0)	Pneumonia. Empyema. Died within 24 hrs.	Confluent lobular pneumonia, R.L., C.; engorgement and edema, R.M., R.U., L.L., L.M., L.U.; serofibrinous pleuritis, bilateral.
125	2,250	" 23	5.0 (" 1.0)	Pneumonia. Empyema. Died within 24 hrs.	Confluent lobular pneumonia, R.U., R.M., R.L., L.L.; engorgement and edema, L.M., L.U.; acute serofibrinous pleuritis, right; acute mediastinitis.
108	2,210	" 29	3.0 (" 1.0)	Pneumonia. Recovered on 4th day.	No autopsy.
123	2,445	" 29	5.0 (" 1.0)	Pneumonia. Killed on 2nd day.	Interstitial and lobular pneumonia, R.L.
128	2,650	" 31	10.0 (" 1.0)	Pneumonia. Empyema. Died within 24 hrs.	Confluent lobular pneumonia, R.U., R.L.; engorgement and edema, R.M.; acute serofibrinous pleuritis, right; chronic fibrous pleuritis, bilateral.
122	3,375	Aug. 6	0.1	Pneumonia; lung abscesses. Killed Aug. 14.	Interstitial pneumonia, R.L.; acute fibrinous pleuritis, right; pulmonary abscesses, R.L.

R.L., R.M., R.U., etc., indicate lobes of the lung.

The clinical features and pathology of hemolytic streptococcus pneumonia in monkeys are presented in the following protocols. The temperature, leucocyte counts, and blood cultures are shown in Text-fig. 1.



TEXT-FIG. 1, *a*, *b*, *c*, and *d*. Clinical course of experimental streptococcus pneumonia following the intratracheal injection of *Streptococcus hæmolyticus*. (*a*) Monkey 120; (*b*) Monkey 119; (*c*) Monkey 126; (*d*) Monkey 128.

Experiment 1.—Monkey 120 (Text-fig. 1, *a*). *Macacus syrichtus*, female; weight 2,800 gm. July 14, 1919. Well and active. July 15, 10.30 a.m. Intratracheal injection of 1 cc. of 18 hour broth culture of *Streptococcus hæmolyticus*. 4 p.m. Appears moderately sick; respirations accelerated. July 16. Moderately sick; respirations accelerated but without expiratory grunt; no cough. July 17.

Condition the same; no definite physical signs of consolidation. July 18. Much improved; breathing quietly. 10.30 a.m. Killed.

Autopsy. Anatomical Diagnosis.—Interstitial pneumonia, left lower lobe; acute fibrinous pleuritis, left; chronic fibrous pleuritis, left.

Pleural cavities.—Contain no fluid; there is fresh fibrin over the posterior surface of the left lower lobe; there are old fibrous adhesions between the visceral and parietal pleuræ on the left; right pleura appears normal. *Lungs.*—The posterior surface of the left lower lobe shows a mottled, deep red area spreading downward from the hilum; the cut surface yields a moderate amount of frothy fluid and presents minute deep red areas apparently of perivascular and peribronchial distribution; there is no firm consolidation; the other lobes appear normal.

Cultures.—Heart's blood and left lower lobe, no growth.

Macroscopic Examination.—Histological section through the left lower lobe (Fig. 1) shows considerable thickening of the vascular adventitia throughout the lobe. This process is particularly conspicuous about the pulmonary veins. There is a less marked thickening of the bronchial walls and interlobular septa in many places. In the upper and inner portion of the lobe there is a wedge-shaped area extending outward from the main bronchus to the pleura in which the alveolar structure appears much thickened, the alveoli atelectatic. There is little or no exudate in the alveoli.

Microscopic Examination.—Left lower lobe.—There is a thin deposit of fibrin on the pleural surface. The pleura is considerably thickened by the formation of new connective tissue, the interstices of which are infiltrated with many large mononuclear cells, plasma cells, lymphocytes, and a few polymorphonuclear leucocytes. The perivascular tissue is everywhere greatly thickened and infiltrated with large mononuclear cells, plasma cells, and lymphocytes, and occasional polymorphonuclear leucocytes (Fig. 2). In many places the interlobular septa and the walls of the bronchi are similarly thickened and infiltrated. The alveolar walls are conspicuously thickened and infiltrated with mononuclear cells and occasional polymorphonuclear leucocytes (Fig. 2), this process being especially prominent in the alveoli which lie adjacent to the blood vessels, bronchi, septa, and pleura. In places there is considerable proliferation of the epithelial cells lining the alveoli, an occasional mitotic figure being seen. The alveoli are for the most part air-containing and in the more extensively involved portions are atelectatic. A few scattered alveoli contain a contracted coagulum in which desquamated epithelial cells and a few leucocytes are embedded (Fig. 2), others contain a dense coagulum without cells, and still others contain a few red blood corpuscles. The bronchi contain little or no exudate, and the epithelium is intact. The epithelium of some of the terminal bronchioles has been desquamated, and the lumina contain a coagulum in which epithelial cells and a few leucocytes are embedded. The lymphatics of the larger bronchi and vessels near the hilum are filled with mononuclear cells. No streptococci are seen. *Bronchial lymph node.*—The marginal sinus is filled with large mononuclear cells and lymphocytes.

Monkey 120 represents a comparatively mild case of pneumonia with recovery on the 4th day. It exhibited no symptoms other than an ill defined appearance of being sick and a moderately accelerated respiratory rate. The clinical chart (Text-fig. 1, *a*) shows a prompt elevation of temperature 6 hours after inoculation with return to normal by rapid lysis, a leucocytosis, which was polymorphonuclear in character, during the acute stage of the disease, and sterile blood cultures throughout. Pathologic examination showed pneumonia in the left lower lobe, the most striking feature of which was its almost entirely interstitial character. The limitation of the process to the framework of the lung would appear to be an expression of a considerable degree of resistance to the infection, a supposition well supported by the brief duration of the disease and the promptness with which the lungs were freed from streptococci. There is no evidence that the disease began as a primary infection of the terminal bronchioles.

Experiment 2.—Monkey 119 (Text-fig. 1, *b*). *Macacus syrichtus*, female; weight 3,180 gm. July 18, 1919. Well and active. July 19, 10.15 a.m. Intratracheal injection of 5 cc. (in 1 cc.) of 18 hour broth culture of *Streptococcus hæmolyticus*. 4.15 p.m. Appears moderately sick; breathing rapidly. July 20. Quiet; respirations accelerated but without expiratory grunt; no cough; slight dullness with diminished breath sounds in right lower axilla; no râles. July 21. Moderately sick; respirations moderately accelerated. July 22. Appears better; apparently recovering; temperature has returned to normal by lysis. 3 p.m. Killed.

Autopsy. Anatomical Diagnosis.—Interstitial and lobular pneumonia, right upper and lower lobes.

Pleural cavities.—Contain no fluid. *Lungs.*—Over the posterior surface of the right lower lobe there is an extensive, pinkish gray area (Fig. 3), more translucent than the rest of the lung and sharply defined from the surrounding pale pink surface. On palpation this area is firm and is found to occupy a considerable part of the substance of the lobe. The cut surface shows an area of consolidation involving the posterior half of the lobe and sharply demarcated from the surrounding tissue. The surface of the consolidated area presents a pinkish gray, translucent appearance, and is slightly moist. There is a similar but smaller area in the right upper lobe. The remainder of the lungs is pale pink and crepitant. Mucosa of the trachea and bronchi appears normal.

Cultures.—Heart's blood and right lower lobe, no growth; right bronchus, *Streptococcus hæmolyticus*.

Microscopic Examination.—Right lower lobe.—The pleura is moderately thickened and infiltrated with large mononuclear cells, lymphocytes, and a few poly-

morphonuclear leucocytes. Young connective tissue cells are beginning to appear. In the pneumonic area the framework of the lung is conspicuously thickened and infiltrated with large mononuclear cells, lymphocytes, plasma cells, and polymorphonuclear leucocytes. This process is especially prominent in the vascular adventitia and is also quite marked in the walls of the bronchi and bronchioles (Fig. 4). The alveolar walls are thickened by swelling and active proliferation of the epithelium which, in places, shows a striking cuboidal appearance, and by moderate infiltration with mononuclear cells and leucocytes (Fig. 5). Nearly all the alveoli contain coagulated serum. In those lying adjacent to the bronchioles and blood vessels there are numerous desquamated epithelial cells, mononuclear phagocytic cells, and polymorphonuclear leucocytes (Fig. 4). In places this exudate may be traced into the lumen of a terminal bronchiole. There are several small areas in which there is considerable necrosis of the alveoli and the exudate is composed largely of polymorphonuclear leucocytes and granular debris. The smaller bronchi contain a moderate amount of exudate, and in places considerable desquamation of epithelium has occurred. No streptococci are seen. *Right upper lobe.*—Presents a similar picture. The process, however, is less extensive and more limited to the interstitial tissue.

Monkey 119 presented a clinical picture quite similar to that of Monkey 120, but appeared somewhat sicker. A temporary invasion of the blood stream by the streptococcus occurred. This probably should not be regarded as anything more than an overflow from the lungs following the inoculation of a large amount (5 cc.) of streptococcus. Autopsy showed a well defined area of interstitial and lobular pneumonia in the right lower lobe and a smaller area in the right upper lobe. Examination of histological sections showed a conspicuous involvement of the framework of the lung similar to that in Monkey 120. In addition, there were areas of lax consolidation, the location of which suggests that the infection had spread outward from the peribronchial and perivascular tissue to the adjacent alveolar structure. The preponderance of mononuclear cell infiltration, the presence of fibroblasts, the active proliferation of alveolar epithelium, and the absence of streptococci indicate that the infection had terminated and that the process of repair had begun.

Experiment 3.—Monkey 126 (Text-fig. 1, *c*). *Macacus syrichtus*, female; weight 1,965 gm. July 19 and 20, 1919. Well and active. July 21, 11 a.m. Intratracheal injection of 10 cc. (in 1 cc.) of 18 hour broth culture of *Streptococcus hæmolyticus*. 4.15 p.m. Appears moderately sick; breathing very rapidly. 11 p.m. Appears very sick; breathing difficult and very rapid. July 22. Died during night.

Autopsy. Anatomical Diagnosis.—Confluent lobular pneumonia, right lower and cardiac lobes; engorgement and edema of right middle and upper, and left lower, middle, and upper lobes; serofibrinous pleuritis, bilateral.

Right pleural cavity.—Contains a considerable quantity of slightly turbid, claret-colored fluid. *Left pleural cavity.*—Contains a smaller amount of similar fluid. *Lungs.*—Voluminous and intensely congested, the surfaces of both lungs being deep pink and thickly mottled with large, dark red, irregular patches. The right lower lobe is fairly firm and nodular on palpation. The cut surface is dark red and oozes a large quantity of claret-colored fluid. On scraping the surface scattered areas of grayish red consolidation are seen. These are elevated and in many cases confluent. The cardiac lobe shows an almost uniform lax consolidation. The cut surface is dark red, moist, and homogeneous. The right middle and upper lobes show intense congestion, the cut surfaces yielding a considerable amount of frothy, blood-tinged fluid, but no definite consolidation is made out. The left lower, middle, and upper lobes present a similar picture. Trachea and bronchi are filled with a bloody, mucoid exudate, but the mucosa appears normal.

Cultures.—Heart's blood, right lower lobe, and right pleural fluid, *Streptococcus hæmolyticus*.

Macroscopic Examination.—Histological section through the right lower lobe shows an intense edema of the vascular adventitia and bronchial walls which are distended to many times their normal thickness. This is most marked about the larger bronchi and vessels. There is intense vascular engorgement. The alveoli lying adjacent to the larger bronchial branches and to the vessels are filled with serous exudate, while those lying midway between and at the periphery are to a considerable extent air-containing. There are several small irregular areas adjacent to some of the larger veins and bronchi where consolidation is present.

Microscopic Examination.—Right lower lobe.—There is a layer of fibrin infiltrated with polymorphonuclear leucocytes on the pleural surface. The pleura is moderately thickened, infiltrated with leucocytes, and in places appears necrotic. The lymphatics of the pleura are greatly distended. In many places they are thrombosed and contain dense masses of streptococci. The bronchial walls and vascular adventitia are greatly distended with edema and infiltrated to a variable degree with polymorphonuclear leucocytes. Dense masses of streptococci are seen in the outer margins of the perivascular tissue. They are also present, though less numerous, in the bronchial walls. The lymphatics of the vessels and bronchi are distended, and are frequently filled with thrombi, leucocytes, or masses of streptococci. The interlobular septa are edematous. The bronchi show epithelial desquamation and in places necrosis of the walls. Many of them contain leucocytes, coagulated serum, and necrotic debris. Streptococci are present in this exudate. The walls of the terminal bronchioles are thickened and infiltrated with leucocytes and streptococci. The epithelium is desquamated. Those in the peripheral portions of the lobe contain no exudate, while many of those in the more central portions where consolidation has occurred

contain leucocytes and necrotic material. In the central portions of the lobe and extending outward along the main bronchial and vascular branches the alveoli are filled with exudate. Those lying adjacent to the bronchi and vessels contain great numbers of polymorphonuclear leucocytes embedded in coagulated serum and some fibrin, those more distant only coagulated serum. The alveolar walls are thin and show no cellular infiltration. In many places there is extensive necrosis of the exudate and alveolar walls. Immense numbers of streptococci are present in the exudate, being particularly numerous in the necrotic areas. In the peripheral portions of the lobe many of the alveoli are air-containing, while others lying adjacent to the vessels, pleura, and bronchioles contain coagulated serum. The walls of the alveoli and atria are edematous, and in places about the terminal bronchioles, alveolar ducts, and vessels are infiltrated with polymorphonuclear leucocytes (Fig. 6). The distribution of streptococci in this portion of the lobe is particularly interesting. Where exudation into the alveoli and bronchioles has not occurred, streptococci are seen in the walls of the terminal bronchioles and in the walls of the adjacent alveoli where they lie between the capillary and the lining epithelium (Fig. 7), but none are seen in the alveolar spaces or in the lumina of the bronchioles. Where serous exudate has poured out into the alveoli streptococci are present in large numbers in the exudate. *Right upper lobe.*—The process is much less advanced and there is no consolidation. The vessels and capillaries are greatly engorged. There is moderate edema of the vascular adventitia and bronchial walls but no cellular infiltration. The interlobular septa are not prominent. There is some desquamation of the bronchial epithelium but the bronchi and bronchioles contain no exudate. The alveoli are air-containing except in a few scattered patches adjacent to the bronchi where they contain a serous exudate. About some of the bronchi and terminal bronchioles the alveolar walls appear moderately edematous. The perivascular and peribronchial lymphatics are distended but contain few cells. Many streptococci are seen on the epithelial surface of a large bronchus and may be seen penetrating the subepithelial layers where they are present in large numbers. In the smaller bronchi and terminal bronchioles of the central portions of the lobe they are present in large numbers in the walls (Fig. 8), but not on the epithelial surface or in the lumen. None are seen in the walls of the peripheral bronchioles. Large numbers are present in the walls of the alveoli that lie adjacent to the bronchi, while the walls of the more distant alveoli are free from organisms. In a similar manner many streptococci are present in the walls of the alveoli in which terminate bronchioles whose walls have been invaded, while the walls of the alveoli in which unaffected bronchioles terminate are free from organisms. Where a serous exudate has poured out into the alveoli streptococci are present in the exudate. In places streptococci are seen in the perivascular lymphatics. At a few points they have reached the pleura but have not spread out in dense masses through the pleural lymphatics.

Experiment 4.—Monkey 125. *Macacus syrichtus*, male; weight 2,250 gm. July 22, 1919. Well and active. July 23, 11 a.m. Intratracheal injection of 5

cc. (in 1 cc.) of 18 hour broth culture of *Streptococcus hæmolyticus*. 4.15 p.m. Quiet; breathing very rapidly; moderate dullness and suppressed breath sounds over right lower lobe. Blood culture shows 1 colony of *Streptococcus hæmolyticus* per 0.5 cc. of blood. 11 p.m. Very sick; respirations difficult and very rapid; evidently suffering from severe pleural pain; crying frequently. July 24. Died between midnight and 9 a.m.

Autopsy. Anatomical Diagnosis.—Confluent lobular pneumonia, right lower, middle, and upper lobes, left lower lobe; engorgement and edema, left middle and upper lobes; acute serofibrinous pleuritis, right; acute mediastinitis.

Anterior mediastinum.—Shows intense congestion and edema of the tissues. *Right pleural cavity.*—Filled with slightly turbid, claret-colored fluid, the right lung being partially collapsed. *Left pleural cavity.*—Contains no fluid. *Lungs.*—The surface of the entire right lung is intensely congested and covered with dark red, blotchy patches, most marked in the middle and lower lobes. The whole lung feels heavy and soggy, with irregular patches of moderately firm consolidation, most marked in the lower lobe. The cut surface of the lower lobe is dark red and shows irregular, patchy, consolidated areas, which become confluent in some places. It yields a large quantity of bloody fluid. The middle and upper lobes present a similar picture, but the consolidation is less marked. The left lung is voluminous, the anterior surfaces being pale pink. Posteriorly the surfaces show irregular patches of a deep red color. On palpation there is an area of moderately firm, nodular consolidation in the upper and posterior portion of the lower lobe. The cut surface yields a considerable amount of bloody fluid and shows a well demarcated area of confluent lobular consolidation in the upper and posterior portion, the surrounding air-containing tissue being intensely congested and edematous. The cut surfaces of the middle and upper lobes are congested and yield a moderate amount of frothy, blood-tinged fluid. Trachea and bronchi are congested and contain a bloody, mucoid exudate.

Cultures.—Heart's blood, right lower lobe, left lower lobe, pleural fluid, and bronchi, *Streptococcus hæmolyticus.*

Macroscopic Examination.—Histological section through the right lower lobe (Fig. 9) shows intense vascular engorgement. There is marked perivascular edema which is particularly conspicuous about the larger vessels. The bronchial walls appear moderately thickened. In the lower half of the lobe there is a fairly large, roughly wedge-shaped area of consolidation extending downward toward the periphery. This area of consolidation merges indefinitely into a surrounding zone of edema which in turn merges into a peripheral zone of alveoli which are largely air-containing. In the upper portion of the lobe there is a similar, though smaller area of consolidation with ill defined margins merging into a surrounding zone of edema. Histological section through the left lower lobe presents a similar picture. Areas of consolidation appear as roughly oval or circular areas surrounding a central vein or bronchus. The margins of the consolidated areas are irregular and merge without sharp demarcation into surrounding zones of edema. The outer and lower portions of the lobe show no consolidation, the alveoli being largely air-containing.

Microscopic Examination.—Right lower lobe.—There is a deposit of fibrin on the pleura. The pleura is edematous and infiltrated with a moderate number of leucocytes and dense masses of streptococci. The lymphatics are distended and contain masses of streptococci, leucocytes, and large mononuclear cells. In many places they are thrombosed. The perivascular tissue is greatly distended with edema, particularly about the pulmonary arteries, but shows very little cellular infiltration (Fig. 10). The interlobular septa are also very edematous. Dense masses of streptococci are seen in the lymphatics and outer layers of the tissue surrounding the pulmonary veins (Fig. 11). The vessels and capillaries are greatly engorged and in many places the latter show thrombi. The bronchial walls are moderately thickened and infiltrated with polymorphonuclear leucocytes and mononuclear cells, and their lymphatics are distended. In places the walls are necrotic and have completely lost their structure. There is extensive desquamation of the epithelium of the bronchi and bronchioles. The larger bronchi and the bronchioles of the central portions of the lobe contain leucocytes and necrotic debris in which many streptococci are seen. The terminal bronchioles of the peripheral portions show desquamation of epithelium, thickening and infiltration of their walls with polymorphonuclear leucocytes and streptococci, but contain no exudate or streptococci. In the central portions of the lobe, particularly in relation to the bronchi and vessels, the alveoli are filled with an exudate of polymorphonuclear leucocytes and small numbers of mononuclear cells embedded in coagulated serum and fibrin (Fig. 10). In places there is also hemorrhage into the alveoli. The alveolar walls are not distended. Streptococci in pairs and short chains are everywhere seen through this exudate. In many areas extensive necrosis with almost complete destruction of the alveolar walls has occurred. Dense masses of streptococci are present in these necrotic areas. Surrounding the areas of consolidation the alveoli show extensive edema and some hemorrhage. In the peripheral portions of the lobe, except immediately adjacent to the pleura, the alveoli are largely air-containing. About the terminal bronchioles and adjacent to the veins their walls are distended with edema and show some cellular infiltration. Streptococci are numerous in the walls of these comparatively intact alveoli, but are not seen in the alveolar spaces. *Cardiac, right middle, and right upper lobes.*—Sections show the same picture though the process is less widespread. A section from the central portion of the right upper lobe shows a point of local injury to the mucosa of a large bronchus (Fig. 12). The epithelium is desquamated, the subepithelial tissue is infiltrated with polymorphonuclear leucocytes, and numerous streptococci are seen invading the bronchial wall (Fig. 13).

Experiment 5.—Monkey 128 (Text-fig. 1, *d*). *Macacus syrichtus*, female; weight 2,650 gm. July 30, 1919. Well and active. July 31, 11.15 a.m. Intratracheal injection of 10 cc. (in 1 cc.) of 18 hour broth culture of *Streptococcus hæmolyticus*. 4.30 p.m. Appears moderately sick; breathing very rapidly. Aug. 1. Died during night.

Autopsy. Anatomical Diagnosis.—Confluent lobular pneumonia, right lower and upper lobes; engorgement and edema, right middle lobe; acute serofibrinous pleuritis, right; chronic fibrous pleuritis, bilateral.

Right pleural cavity.—Contains a moderate amount of slightly turbid, claret-colored fluid. *Left pleural cavity.*—Contains no fluid. There are scattered old fibrous adhesions in both pleural cavities. *Lungs.*—The right lung is covered with old fibrous tags and the lobes are bound together by old fibrous adhesions. The surface of the upper lobe is congested and marked at many points by small dark red blotches. The cut surface appears congested and yields a considerable amount of blood-tinged fluid. There is some peribronchial consolidation. The middle and lower lobes are intensely congested and feel heavy and soggy. On palpation the lower lobe shows a moderately firm, nodular area of consolidation in the lower portion. The cut surfaces of the middle and lower lobes are dark red and ooze a large quantity of bloody fluid. In the lower lobe there is an irregular patch of consolidation in the lower portion, dark red in color, and somewhat elevated above the surrounding edematous and air-containing tissue. The left lung appears moderately congested but is everywhere air-containing and free from consolidation.

Cultures.—Heart's blood, pleural fluid, and right lower lobe, *Streptococcus hæmolyticus.*

Microscopic Examination.—Right lower lobe.—The pleura is greatly thickened by old fibrous tissue, the interstices of which are infiltrated with polymorphonuclear leucocytes, mononuclear cells, and moderate numbers of streptococci. The pleural lymphatics are greatly distended and everywhere contain dense masses of streptococci. The vessels are greatly engorged and many of the smaller vessels and capillaries contain thrombi. The perivascular tissue is edematous and infiltrated with leucocytes. Dense masses of streptococci are present in the distended lymphatics and outer layers of the adventitia of the pulmonary veins (Fig. 14). The bronchial walls are thickened and infiltrated with leucocytes and streptococci and in places are completely necrotic. The epithelium is desquamated. The walls of the terminal bronchioles are similarly thickened and infiltrated. The interlobular septa are edematous, infiltrated with polymorphonuclear leucocytes, and their lymphatics are distended and filled with streptococci, leucocytes, and thrombi. Except at the peripheral portions the alveoli are edematous and contain many disintegrating leucocytes. Much of the tissue is undergoing necrosis, the structure of the alveolar walls being indistinct (Fig. 14). Streptococci are abundant in these areas. At the periphery the involvement is largely interstitial without exudation into the alveoli. The alveolar walls are greatly distended with edema, and infiltrated with leucocytes, and contain abundant streptococci which, however, are not seen in the alveolar spaces.

Monkeys 126, 125, and 128 illustrate very severe and rapidly fatal cases of streptococcus pneumonia associated with the early develop-

ment of serofibrinous pleurisy. The extremely rapid respiratory rate exhibited by these monkeys was undoubtedly due to the rapid accumulation of a large pleural exudate. The almost negligible invasion of the blood stream by the streptococcus 6 hours after inoculation, in spite of the overwhelming nature of the infection and the large numbers of streptococci injected, is striking. Monkeys receiving much smaller intratracheal injections of pneumococcus invariably showed a very heavy septicemia 6 hours after inoculation. It is noteworthy that none of these monkeys, though very severely infected, showed any evidence of prostration during the onset of the pneumonia, exhibiting no definite symptoms other than the rapid respiratory rate and an ill defined appearance of being sick, yet all died within 24 hours after inoculation.

The pathologic picture was one of rapid and widespread invasion of the lungs with little show of resistance on the part of the tissue. Proliferation of the alveolar epithelium, extensive mononuclear cell reaction, and limitation of the process to the interstitial tissue were lacking. The primarily interstitial nature of the infection, though somewhat masked by the widespread confluent areas of alveolar edema, consolidation, and necrosis, was, nevertheless, shown by the intense perivascular edema and leucocytic infiltration, the thickening and infiltration of the walls of the bronchi and bronchioles, and the edema of the interlobular septa. It was even more strikingly shown in the peripheral areas of the lung where the process was entirely interstitial in character without exudation into the alveolar spaces and lumina of the bronchioles. Since these were all cases of less than 24 hours duration the distribution of streptococci is of particular significance in indicating the mode of invasion of the lung. In the earliest lesion observed (Monkey 126, right upper lobe) streptococci are present on the epithelial surface of a large central bronchus, and where the epithelium is injured they may be seen penetrating into the peribronchial tissue. This is also well shown in a section from the right upper lobe of Monkey 125 (Fig. 13). In the smaller bronchi and bronchioles, however, they are seen only in the walls, and none are present in the lumina or on the epithelial surfaces. They are also present in the walls of alveoli that lie immediately adjacent to the bronchi and bronchioles whose walls are infected, but are not seen

elsewhere in the alveolar structure. A few are seen in the perivascular lymphatics, and in a few localized spots they have reached the pleura where they lie in masses in the pleural lymphatics. In the more advanced lesions they are present, in addition to the areas described above, in dense masses in the lymphatics about the pulmonary veins, in the interlobular septa, and in the pleura. Wherever exudate has poured out into the alveoli abundant streptococci are present in the alveoli, being particularly numerous in areas that are undergoing necrosis. But where exudation into the alveoli has not occurred the streptococci are confined within the alveolar walls, lying between the central capillary and the epithelial lining of the air space.

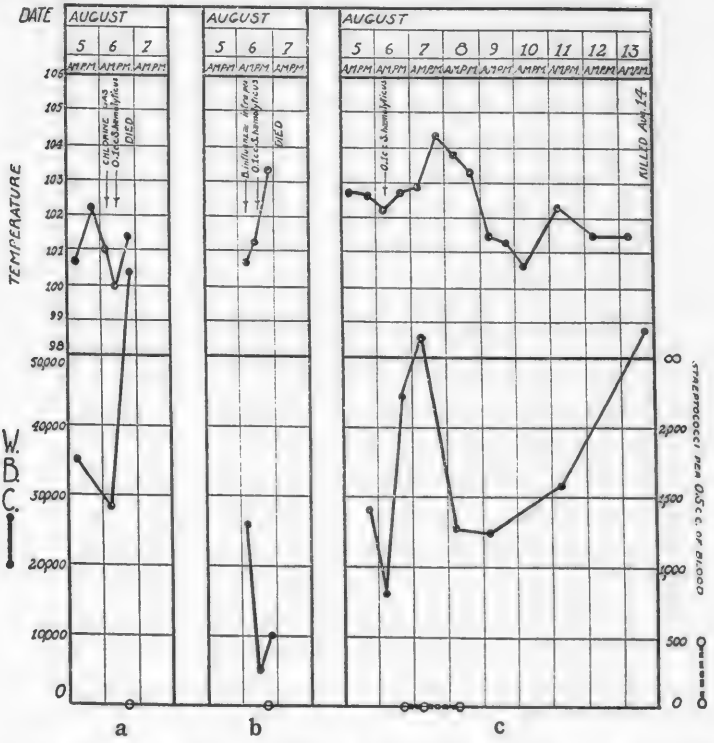
Effect of Preceding Injury to the Respiratory Tract and of Lowering of General Resistance on the Course of Experimental Streptococcus Pneumonia.

The preceding experiments have shown that the normal monkey is comparatively resistant to intratracheal injections of a highly virulent hemolytic streptococcus and succumbs only when large doses are employed. As has been pointed out, such a result is not out of harmony with the infrequent occurrence of primary streptococcus pneumonia in man, in whom the disease commonly occurs only as a secondary infection following some preceding disease affecting the respiratory tract.

It seemed of interest, therefore, to determine the effect of a preceding local injury to the respiratory tract and of the lowering of general resistance without local injury to the respiratory tract on the course of streptococcus pneumonia subsequently produced by the intratracheal injection of streptococci.

Experiment 6.—Four monkeys were used. Preliminary injury to the respiratory tract was produced in Monkey 19 by a few seconds exposure to chlorine gas 1 hour before intratracheal injection of *Streptococcus hæmolyticus*, in Monkey 129 by the intratracheal injection of 0.000001 cc. of Pneumococcus Type I 24 hours before intratracheal injection of streptococcus. Lowering of general resistance without injury to the respiratory tract was produced in Monkey 48 by the intraperitoneal injection of a strain of *B. influenzæ* possessing pathogenicity for monkeys 3½ hours before intratracheal injection of streptococcus. A normal monkey, No. 122, served as a control, receiving only an intratracheal injection of

streptococcus. A small amount of streptococcus, 0.1 cc., was injected in all the animals. The essential data of the experiment are shown in Table II and Text-figs. 2 and 3.



TEXT-FIG. 2, *a*, *b*, and *c*. Effect of preceding inhalation of chlorine gas (Monkey 19) and of intraperitoneal injection of *B. influenzæ* (Monkey 48) on the course of experimental streptococcus pneumonia produced by intratracheal injection of 0.1 cc. of broth culture of *Streptococcus hæmolyticus*. (*a*) Monkey 19; (*b*) Monkey 48; (*c*) Monkey 122, control.

The foregoing experiment shows that in the presence of a local injury to the respiratory tract caused by gassing with chlorine a small amount of *Streptococcus hæmolyticus*, which in a normal control monkey produced only a mild pneumonia localized in one lobe, produced a rapid and widespread infection of the lungs. It is, of course,

TABLE II.

Effect of Preceding Injury to the Respiratory Tract and of Lowering of General Resistance on the Course of Hemolytic Streptococcus Pneumonia.

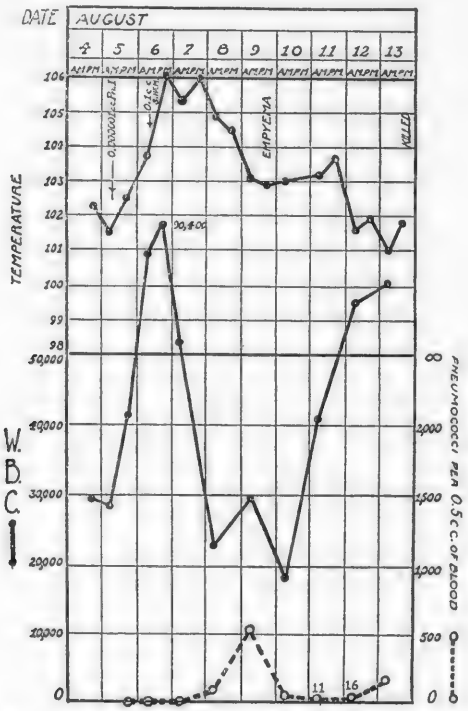
Monkey No.	Weight.	Preliminary procedures.	Condition at time of intratracheal injection of *S. hæmolyticus*.	Time of intratracheal injection of 0.1 cc. of *S. hæmolyticus*.	Result.	Autopsy.	
						Macroscopic pathology.	Cultures.
	gm.	*1919*		*1919*			
19	4,000	Aug. 6, 10 a.m. Exposed for a few seconds to dilute chlorine gas.	Coughing, but otherwise appears well.	Aug. 6, 11 a.m.	Rapidly progressive pneumonia. Aug. 6, 5.30 p.m. Died.	Widespread confluent lobular pneumonia, all lobes, stage of engorgement and edema.	H.B., n.g. Br., S.H. Lungs, S.H.
48	4,785	Aug. 6, 10.15 a.m. Intraperitoneal injection of 13 cc. of salt solution suspension of *B. influenzæ*.	Lying prostrate; abdomen tense and tender; marked leucopenia.	Aug. 6, 1.50 p.m.	Rapidly progressive pneumonia. Aug. 7. Died.	Widespread confluent lobular pneumonia, R.L., R.M., R.U., L.M.; acute serofibrinous pleuritis, right; acute general peritonitis.	H.B., S.H., B.I. R.L., " Pl., " Tr., " Per., B.I., S.H.
129	2,530	Aug. 5, 11.15 a.m. Intratracheal injection of 0.000001 cc. of Pn. I.	Has developed lobar pneumonia; moderately sick.	Aug. 6, 10.35 a.m.	Lobar pneumonia. Developed empyema on 5th day. Aug. 13. Killed.	Lobar pneumonia, R.L., beginning resolution and organization; empyema, right.	H.B., Pn.I. Tr., " I. R.L., " I. Emp., " I.

122	3,375	None (control).	Well and active.	Aug. 6, 10.45 a.m.	Mild pneumonia with recovery by lysis on 4th day. Aug. 14. Killed.	Interstitial pneumonia, R.L.; acute fibrinous pleuritis, right; pulmonary abscesses, R.L.	H.B., n.g. Abscess, S.H.

R.L., R.M., R.U., etc., indicate lobes of the lung.

H.B. indicates heart's blood; Br., bronchus; Pl., pleural fluid; Tr., trachea; Per., peritoneum; n.g., no growth in cultures; S.H., *Streptococcus hæmolyticus*; B.I., *Bacillus influenzæ*; Pn. I, Pneumococcus Type I.

clear that chlorine poisoning was in large part the cause of the rapidly fatal outcome in Monkey 19, and it is not intended to imply that death was due to streptococcus pneumonia. It seems quite clear, nevertheless, that the pulmonary injury produced by chlorine greatly facilitated invasion of the lungs by the streptococcus.



TEXT-FIG. 3. Monkey 129. Effect of the intratracheal injection of 0.1 cc. of broth culture of *Streptococcus hæmolyticus* during the course of experimental Pneumococcus Type I pneumonia.

In a similar manner a widespread streptococcus pneumonia with pleural exudate developed in Monkey 48 which had previously received an intraperitoneal injection of *Bacillus influenzæ* sufficiently large to cause extreme prostration and marked leucopenia at the time streptococcus was injected into the trachea. Since there was no

preceding local injury to the respiratory tract in this case, other factors must have contributed to lack of resistance against streptococcus invasion of the lungs. It would seem probable that the destruction of leucocytic defense as evidenced by the marked leucopenia and by the almost entire absence of polymorphonuclear leucocytes in the pulmonary exudate (Fig. 15) played a considerable part. Whether other factors were concerned it is impossible to say.

Regarding Monkey 129 there is no certain evidence that the intratracheal injection of streptococcus in the presence of a pneumococcus pneumonia exerted any effect on the course of the disease. The early development of empyema during the course of the pneumococcus pneumonia is unusual, and suggests that the empyema may have been initiated by *Streptococcus hæmolyticus*. Failure to find streptococci in the empyema fluid at autopsy, however, throws considerable doubt on this assumption. Likewise, though study of histological sections from the lung suggested that the streptococcus was in part responsible for the lesion, there is no bacteriological evidence in support of this view, and the matter must remain in doubt.

The control monkey, No. 122, which had only a mild pneumonia largely of the interstitial type (Figs. 16 to 19) showed an important feature in the development of pulmonary abscesses (Fig. 16) in which innumerable streptococci were present.

Effect of Inoculating a Monkey in the Nose and Throat with Streptococcus hæmolyticus.

It has been shown[1] that normal monkeys may receive a large inoculation of virulent Pneumococcus Type I in the nose and throat without suffering any apparent ill effect. It seemed desirable, therefore, to determine the effect of *Streptococcus hæmolyticus* in this respect.

Experiment 7.—Monkey 135. *Macacus syrichtus*, male; weight 2,150 gm. Aug. 19, 1919. Well and active. 9.20 p.m. 2.5 cc. of 10 hour broth culture of *Streptococcus hæmolyticus* sprayed into nose and throat. Aug. 20 to 31. Remained well and active with no evidence of infection. Temperature and leucocyte count remained normal.

The above experiment shows that a virulent hemolytic streptococcus inoculated into the nose and throat of a normal monkey failed

to produce pneumonia. Furthermore, like the pneumococcus, it failed to initiate an infection of the upper respiratory tract or exert any apparent effect whatsoever.

DISCUSSION.

The foregoing experiments have shown that pneumonia produced in monkeys by the intratracheal injection of *Streptococcus hæmolyticus* differs in many respects, both clinically and pathologically, from pneumonia produced by the pneumococcus.[1,2] The differences exhibited appear to be quite similar to those which occur in man between hemolytic streptococcus pneumonia and pneumococcus lobar pneumonia.

Clinically, except in the animals which developed empyema, the disease was comparatively mild and was characterized by an inconstant type of temperature curve of 3 to 5 days duration with recovery by lysis. The respirations, though accelerated, were not characterized by the expiratory grunt commonly seen in lobar pneumonia in monkeys. Failure of the streptococcus to invade the blood stream except as a terminal event in the fatal cases was in striking contrast with the constant and early invasion of the blood by the pneumococcus. In all the above features the disease appears to be similar to streptococcus pneumonia in man. It was even more strikingly so in its complications and pathology. In four out of eleven cases (36.3 per cent) the early development of an abundant serofibrinous pleural exudate teeming with streptococci took place. In contrast with this, empyema developed but once (1.6 per cent) in 61 monkeys with experimental pneumococcus lobar pneumonia, and then only late in the disease. One monkey developed mediastinitis, one pulmonary abscesses, both of which conditions occur as complications of streptococcus pneumonia in man.

Particular interest attaches to the pathology of hemolytic streptococcus pneumonia because of the many detailed studies of the disease that have been made during the epidemics of streptococcus pneumonia of 1917 and 1918.

The views of MacCallum,[4] who has made an extensive study of the subject, may be summarized briefly. Two main types of pulmonary lesions are found, interstitial bronchopneumonia and lobular pneumonia. These two types may be, and frequently are combined in the same case. In both instances the infection is a descending one from the upper respiratory tract. In the interstitial type the pneumonia begins as a primary infection of the bronchioles which leads to great changes in their walls and in the adjacent pulmonary tissue with the formation of firm nodules of consolidation about the terminal portions of the bronchi. In the course of the affection thickening and infiltration of the bronchial and alveolar walls, of the interlobular septa, of the perivascular adventitial tissue, and of the pleura occur. The lymphatics of these structures are widely infected and thrombosed. The process is limited in large part to the framework of the lung. The picture of rapid organization and mononuclear cell reaction indicates the tendency of the lung to heal by barricading the further invasion of the infecting organisms. Streptococci are found in large numbers in the bronchial exudate, in the thrombosed lymphatics, and in the pleura, but few are present in the lung substance.

The second type, or lobular pneumonia, is composed of cases in which the streptococci invade in the same way. Little resistance is offered to the infection, however, and large patches of confluent lobular consolidation occur in which the alveoli are filled with blood, leucocytes, and enormous numbers of streptococci. The interstitial and mononuclear cell reaction, which are expressions of resistance, are wanting, and widespread areas of necrosis may occur. There are many cases in which this lobular pneumonia occurs in the pure form, but there are many others in which it is found side by side with the interstitial bronchopneumonia. They are different expressions of reaction to the same infection, the interstitial type being an expression of marked resistance, the lobular type one of feeble resistance.

It seems quite clear that the pneumonia produced in monkeys by *Streptococcus hæmolyticus* is essentially identical in its pathology with that which occurs in man. Furthermore, MacCallum's suggestion that the occurrence of interstitial pneumonia or lobular pneumonia or combinations of the two types depends upon the resistance offered to the infection would seem to be well supported by the results of our experiments, since with increasing doses of streptococci the pathologic picture passed from that of a purely interstitial pneumonia to that of a mixed interstitial and lobular pneumonia, and finally with very

[4] MacCallum, W. G., The pathology of the pneumonia in the United States Army camps during the winter of 1917–18, Monograph of The Rockefeller Institute for Medical Research, No. 10, New York, 1919.

large doses to that of a widespread confluent lobular pneumonia with necrosis.

There is, however, one important difference between the picture presented in monkeys and that described by MacCallum in man; namely, the site of the initial lesion and the primary mode of invasion of the lung by the streptococcus. MacCallum states that the initial lesion is one of the terminal bronchioles and that in the course of the affection thickening and infiltration of the bronchial and alveolar walls, of the interlobular septa, of the perivascular adventitial tissue, and of the pleura occur, the streptococcus invading by way of the lymphatics of these structures. In the disease in monkeys, on the other hand, the initial lesion, or point of invasion of the streptococcus, appears to be at one or more points in the walls of the larger or medium sized bronchi, with extension of the infection from these points through the lung by way of the lymphatics of the bronchial walls to the bronchioles and of the perivascular tissue and interlobular septa to the pleura. Study of early lesions would seem to indicate that infection of the alveoli is primarily by invasion of the alveolar walls by streptococci which spread out during the progress of the infection of the framework of the lung to the alveolar walls that lie adjacent to these structures. Injury of the walls and desquamation of the epithelium of the bronchioles and smaller bronchi with exudation of leucocytes into the lumen would appear to be secondary to invasion of the walls of the bronchioles and smaller bronchi by streptococci which have reached these points by way of the peribronchial lymphatics rather than by way of the bronchial lumen. In a similar manner exudation into the alveoli follows interstitial invasion of the alveolar walls and consequent injury to them. The constant tendency of the tissue to barricade the progress of the infection brings about the characteristic limitation of the alveolar lesions to the alveoli which lie adjacent to the bronchial and vascular trees, the septa, and the pleura, and only when this resistance gives way does a rapidly spreading confluent lobular pneumonia develop. It is, of course, possible that during the progress of the invasion of the peribronchial lymphatics no great injury to the surrounding alveolar tissue may be produced until the streptococci attain the lymphatics of the bronchioles, in which case the disease might appear as a primary infection of the bronchioles

as described by MacCallum. On the other hand, were it actually a primary infection of the bronchioles, it is difficult to correlate this conception with the very early and widespread infection of the perivascular tissue, interlobular septa, and pleura, which occurs even in areas where the terminal bronchioles and their adjacent alveoli remain unaffected. In this connection it should be remembered that the primary mode of invasion and the earliest lesions which may be easily studied in experimental animals, can rarely be studied in man, but must be inferred to a considerable extent from the picture found at later stages of the disease, and that in man the pathology of streptococcus pneumonia is frequently complicated by a preceding and often concomitant bronchial infection with other organisms.

It will be seen from the foregoing discussion that the mode of invasion of the lung by *Streptococcus hæmolyticus* is essentially identical with the mode of invasion by the pneumococcus as described in a preceding paper on the pathogenesis of lobar pneumonia.[2] It is furthermore apparent that the first response of the animal to this invasion is the same in both cases; namely, vascular engorgement and interstitial edema with polymorphonuclear leucocytic infiltration. Following the stage of lymphatic and interstitial invasion, however, the end-results are divergent. In pneumococcus lobar pneumonia little resistance is offered by the tissue to the invading organisms and the process sweeps rapidly through the lobe, ultimately producing a comparatively homogeneous complete lobar consolidation without necrosis or extensive damage to the bronchi. In streptococcus interstitial pneumonia on the other hand, an almost immediate resistance to the invading organisms is offered, the process is limited in large part to the interstitial tissue and lymphatics of the pulmonary framework and to the alveoli immediately adjacent, and the picture of active repair is encountered. If, on the other hand, comparatively little resistance is offered to the infection, the process sweeps through the lobe, producing a confluent lobular pneumonia which ultimately may lead to consolidation of an entire lobe. In this respect the disease is analogous to lobar pneumonia. There is, however, a striking difference between the two which would appear to depend upon the power of the streptococcus to produce marked local injury to the tissues, resulting in extensive damage to the walls of the bronchi

and in widespread areas of alveolar necrosis, features which are not often seen in pneumococcus pneumonia. The greater tendency for extensive organization to occur following streptococcus pneumonia is another feature in which the two diseases differ. It would seem possible that this difference may be dependent upon the more extensive tissue injury caused by the streptococcus than by the pneumococcus.

SUMMARY.

1. Pneumonia has been consistently produced in normal monkeys by intratracheal injection of *Streptococcus hæmolyticus*.

2. The pneumonia produced has been shown to be comparable with hemolytic streptococcus pneumonia in man with respect to its clinical features, complications, and pathology.

3. Two pathologic types of the disease have occurred, interstitial pneumonia and confluent lobular pneumonia. Both types have been found in the same animal.

4. The type of pneumonia has appeared to be dependent upon the amount of streptococcus culture injected, interstitial pneumonia following the injection of small amounts and being an expression of considerable resistance, confluent lobular pneumonia following the injection of large amounts and being an expression of comparative lack of resistance.

5. Study of the distribution of streptococci in the lungs and of the character of the lesions in early stages of the disease has shown that streptococci may primarily invade the pulmonary tissue by penetration of the walls of the larger bronchial branches and that they are distributed from the points of invasion by way of the peribronchial, perivascular, and septal interstitial tissue and lymphatics. Infection of the alveoli is likewise primarily an interstitial invasion of the alveolar walls by streptococci.

6. In one experiment it was found that preliminary injury to the respiratory tract by gassing with chlorine and that lowering of resistance by a preceding intraperitoneal injection of *Bacillus influenzæ* without local injury to the respiratory tract greatly facilitated invasion of the lungs by *Streptococcus hæmolyticus*.

7. A normal monkey inoculated in the nose and throat with *Streptococcus hæmolyticus* failed to develop pneumonia and showed no evidence of infection of the upper respiratory tract.

CONCLUSIONS.

1. *Streptococcus hæmolyticus* can produce a primary pneumonia in monkeys when injected intratracheally in sufficiently large amounts.

2. It readily produces an extensive secondary pneumonia in monkeys when injected intratracheally in small amounts.

3. Invasion of the lungs by *Streptococcus hæmolyticus* in streptococcus pneumonia in monkeys is primarily by way of the interstitial framework of the lung and its lymphatics, and the disease does not appear to be primarily an infection of the terminal bronchioles.

4. Although it seems probable that invasion of the lungs by *Streptococcus hæmolyticus* in streptococcus pneumonia in man may be by the same paths, it is unsafe to draw this conclusion without qualification, since streptococcus pneumonia in man commonly occurs only as a secondary infection in the presence of a preceding inflammatory bronchitis.

EXPLANATION OF PLATES.

PLATE 35.

FIG. 1. Monkey 120. Interstitial pneumonia. Histological section completely through the left lower lobe showing limitation of the lesions to the interstitial framework of the lung. × 3.5.

FIG. 2. Monkey 120. Interstitial pneumonia. Infiltration of perivascular tissue and alveolar walls. × 100.

PLATE 36.

FIG. 3. Monkey 119. Interstitial and lobular pneumonia. Posterior aspect of the lungs.

FIG. 4. Monkey 119. Interstitial and lobular pneumonia. Section from the right lower lobe showing exudate in alveolar duct and terminal bronchiole with epithelial desquamation, perivascular infiltration, and exudation into alveoli. × 100.

PLATE 37.

FIG. 5. Monkey 119. Interstitial and lobular pneumonia. Proliferation and desquamation of alveolar epithelium. × 400.

PLATE 38.

FIG. 6. Monkey 126. Confluent lobular pneumonia. Section from the periphery of the right lower lobe showing edema and infiltration of the walls of atrium and adjacent alveoli with no exudate in the atrium or alveoli. × 100.

FIG. 7. Monkey 126. Confluent lobular pneumonia. Streptococci in an alveolar wall in an unconsolidated portion of the lobe. × 1,000.

PLATE 39.

FIG. 8. Monkey 126. Confluent lobular pneumonia. Early lesion of the right upper lobe showing streptococci in the wall of a terminal bronchiole. × 1,000.

FIG. 9. Monkey 125. Confluent lobular pneumonia. Histological section through the right lower lobe. × 3.5.

PLATE 40.

FIG. 10. Monkey 125. Confluent lobular pneumonia. Periarterial edema and alveolar consolidation. × 100.

FIG. 11. Monkey 125. Confluent lobular pneumonia. Dense masses of streptococci in perivascular tissue. × 100.

PLATE 41.

FIG. 12. Monkey 125. Confluent lobular pneumonia. Early lesion in the right upper lobe showing a point of injury to the epithelium of a large bronchus and leucocytic infiltration and necrosis of the bronchial wall at this point. × 100.

FIG. 13. Monkey 125. Confluent lobular pneumonia. Streptococci in the lesion of the wall of the large bronchus shown in Fig. 12. × 1,000.

PLATE 42.

FIG. 14. Monkey 128. Confluent lobular pneumonia. Section from the right lower lobe showing necrosis and dense masses of streptococci about two small vessels. × 100.

FIG. 15. Monkey 48. Confluent lobular pneumonia. Section showing capillary engorgement, perivascular edema, and serous exudate in alveoli with complete absence of polymorphonuclear leucocytes due to leucopenia following a preliminary intraperitoneal injection of *B. influenzæ*. × 100.

PLATE 43.

FIG. 16. Monkey 122. Interstitial pneumonia. Section through the right lower lobe showing two pulmonary abscesses. × 3.5.

FIG. 17. Monkey 122. Interstitial pneumonia. Organization and mononuclear cell infiltration of bronchial wall. × 100.

PLATE 44.

FIG. 18. Monkey 122. Interstitial pneumonia. Infiltration and organization of interlobular septum and perivascular tissue. × 100.

FIG. 19. Monkey 122. Interstitial pneumonia. Organization of exudate. × 100.

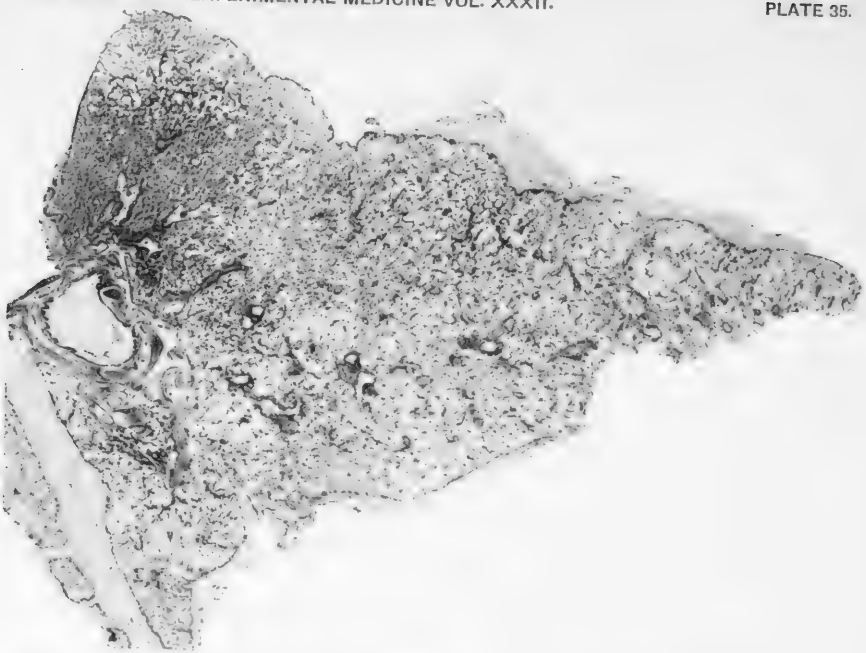


FIG. 1.

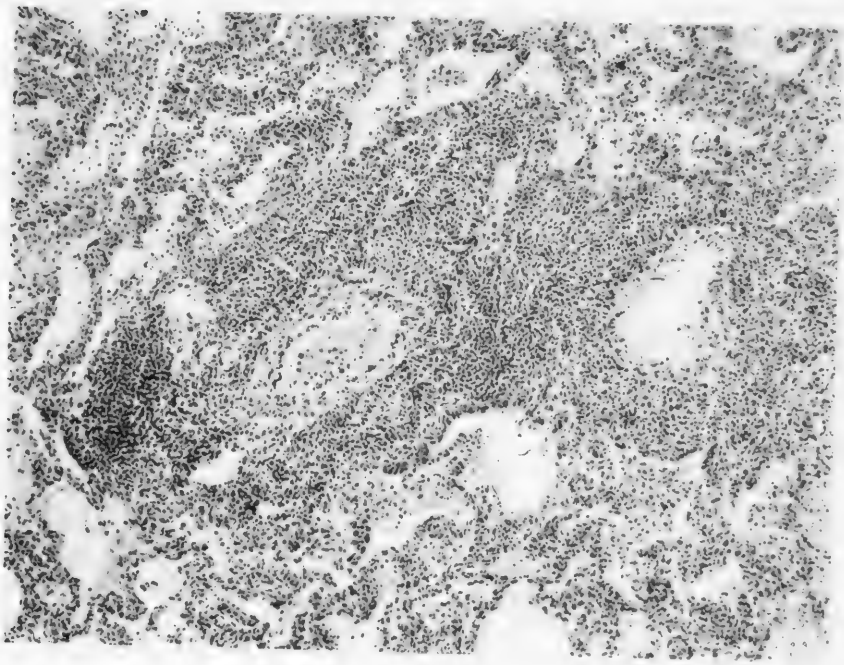


FIG. 2.

(Blake and Cecil: Experimental pneumonia. VIII.)

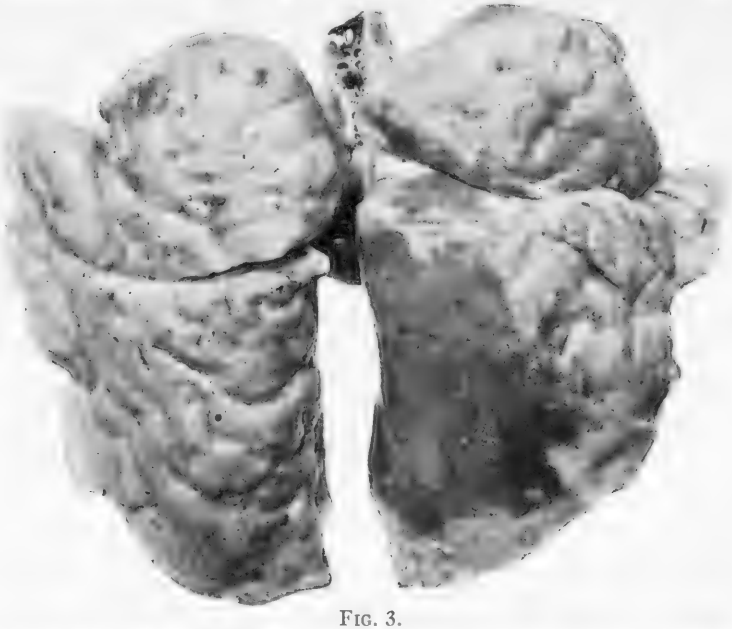


FIG. 3.

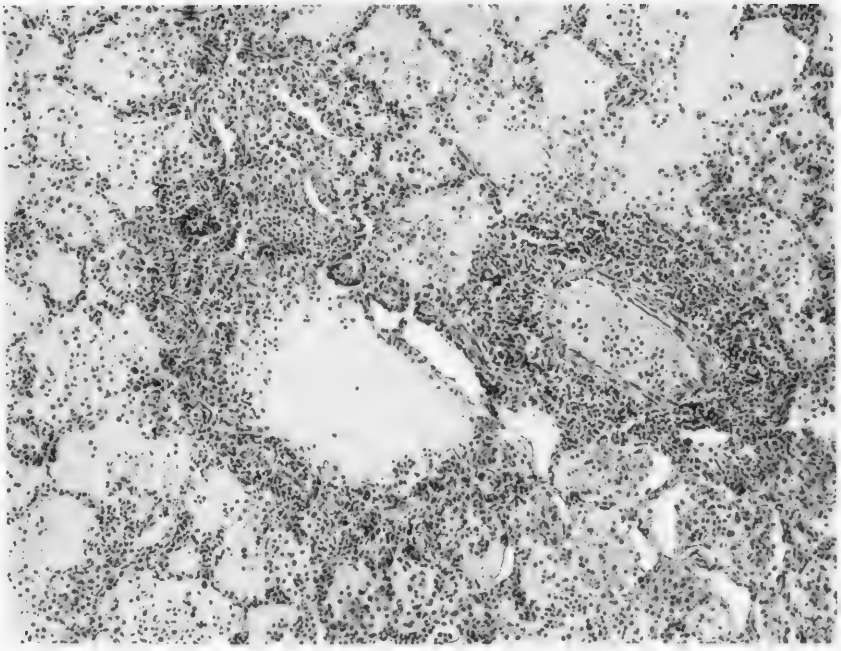


FIG. 4.

(Blake and Cecil: Experimental pneumonia. VIII.)

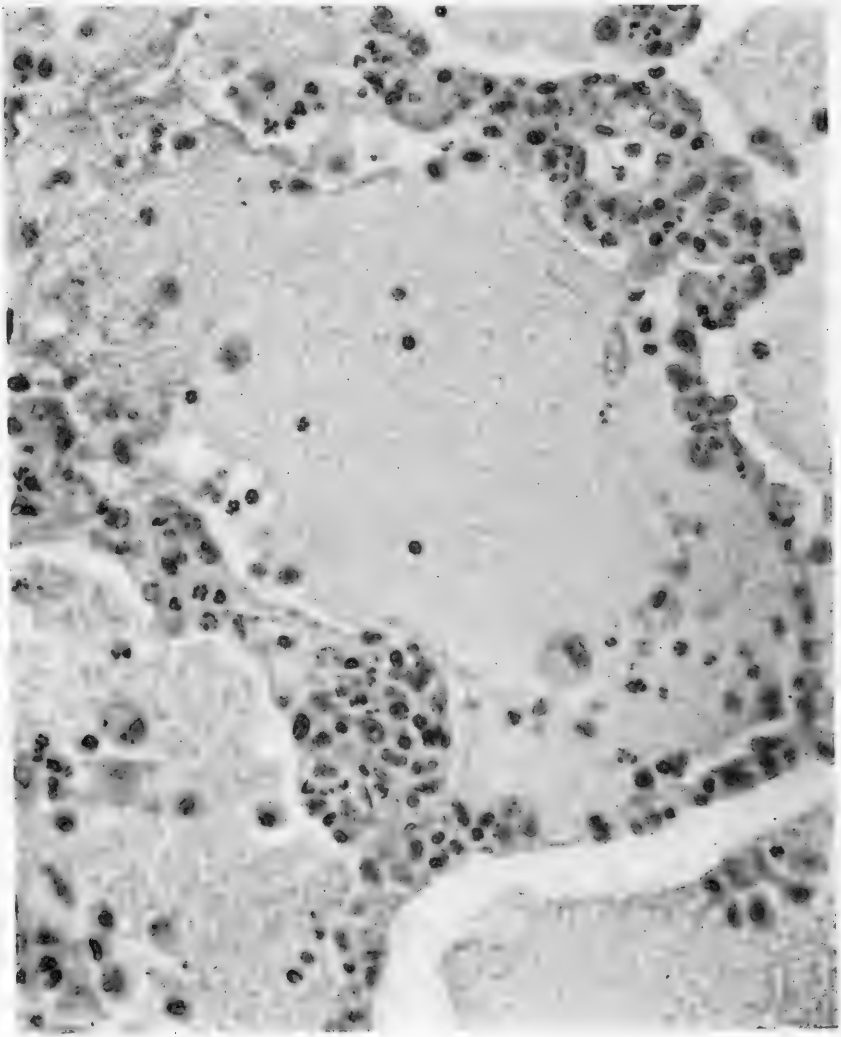


FIG. 5.

(Blake and Cecil: Experimental pneumonia. VIII.)

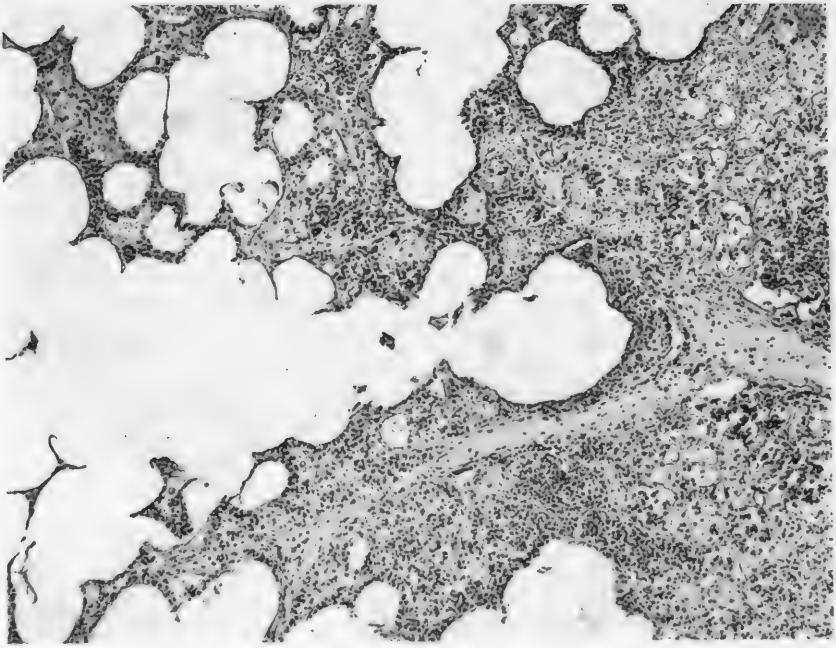


FIG. 6.

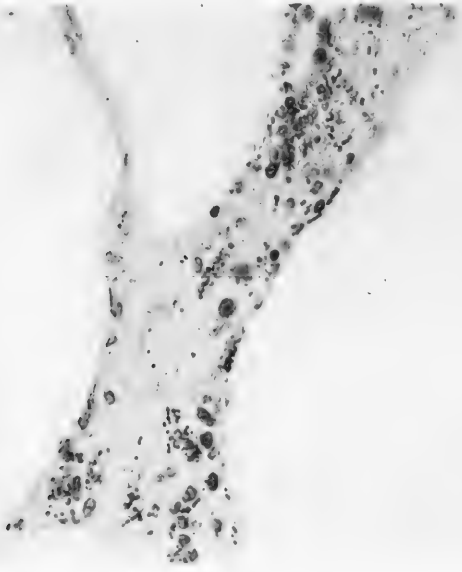


FIG. 7.

(Blake and Cecil: Experimental pneumonia. VIII.)

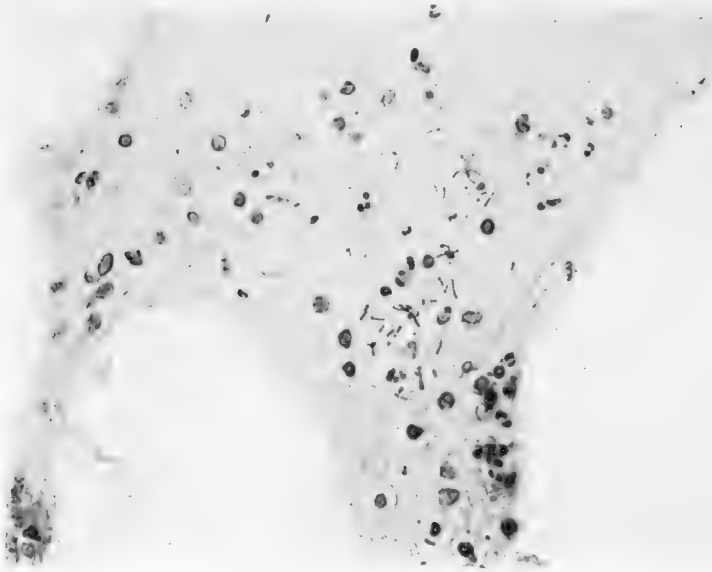


FIG. 8.

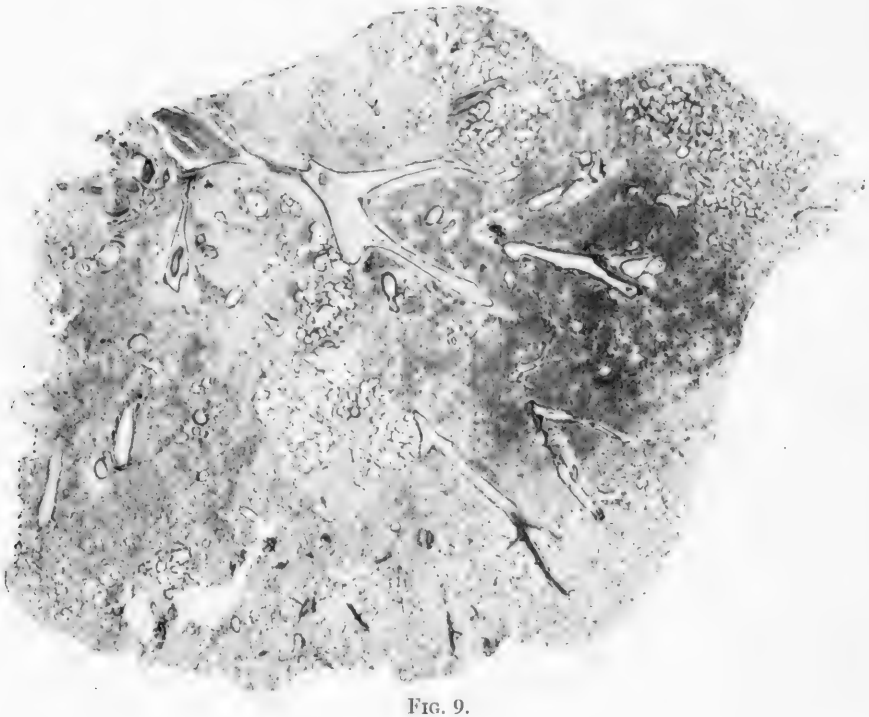


FIG. 9.

(Blake and Cecil: Experimental pneumonia. VIII.)

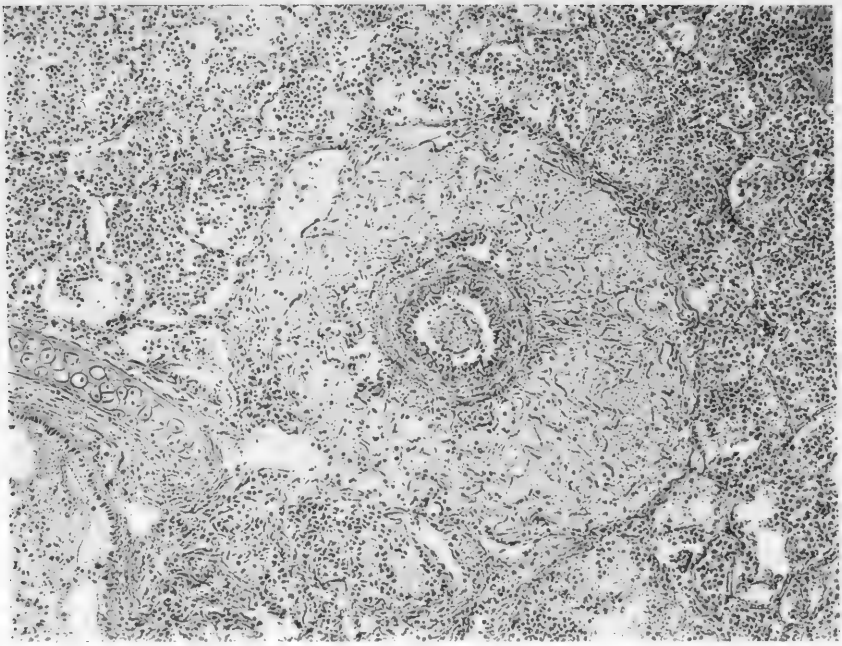


FIG. 10.

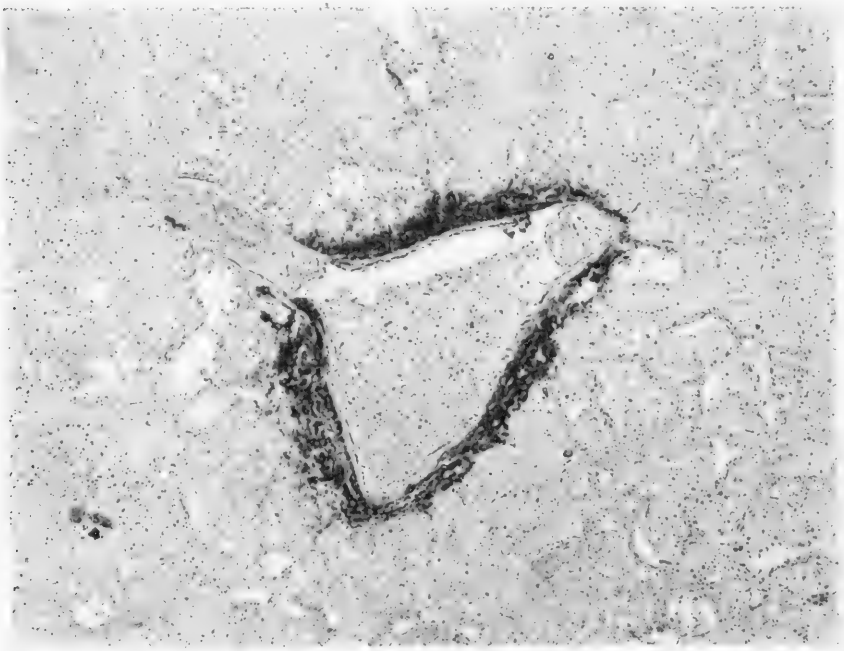


FIG. 11.

(Blake and Cecil: Experimental pneumonia. VIII.)

FIG. 12.

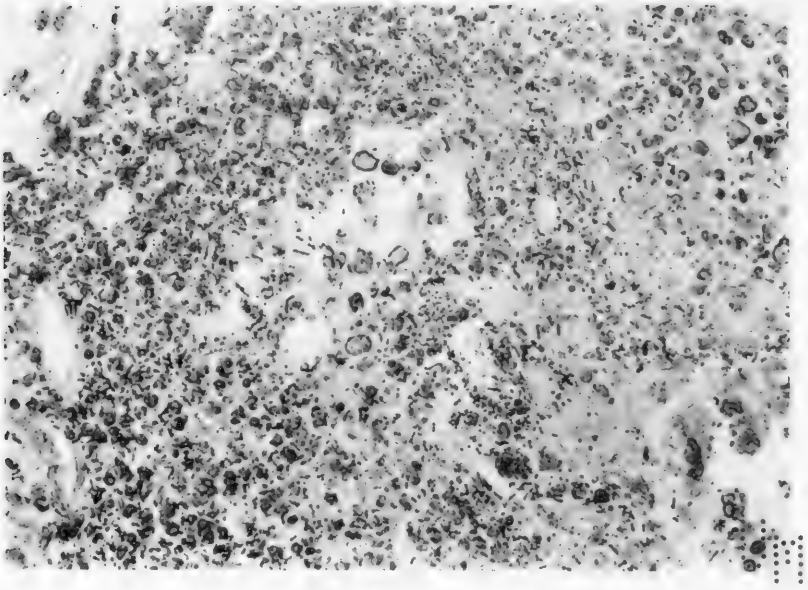


FIG. 13.

(Blake and Cecil: Experimental pneumonia. VIII.)

FIG. 14.

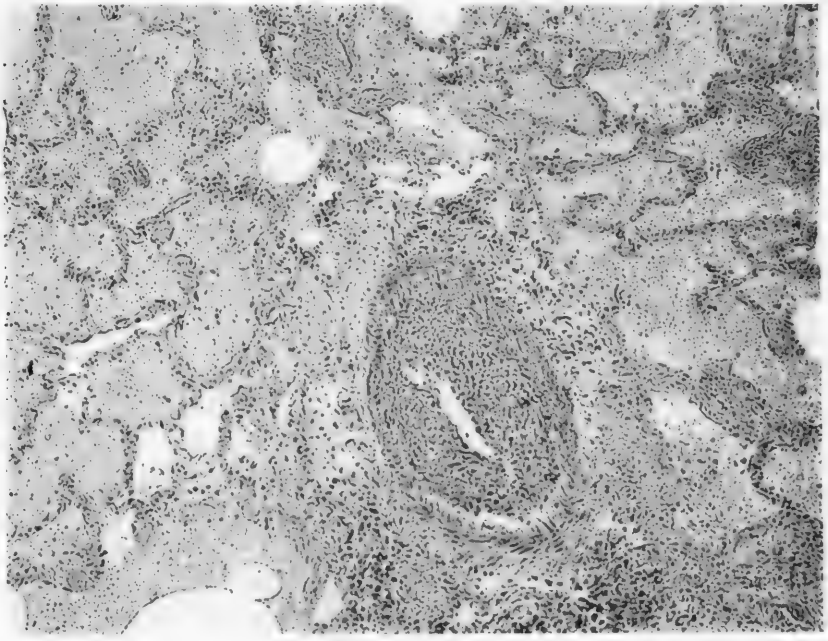


FIG. 15.

(Blake and Cecil: Experimental pneumonia. VIII.)

FIG. 16.

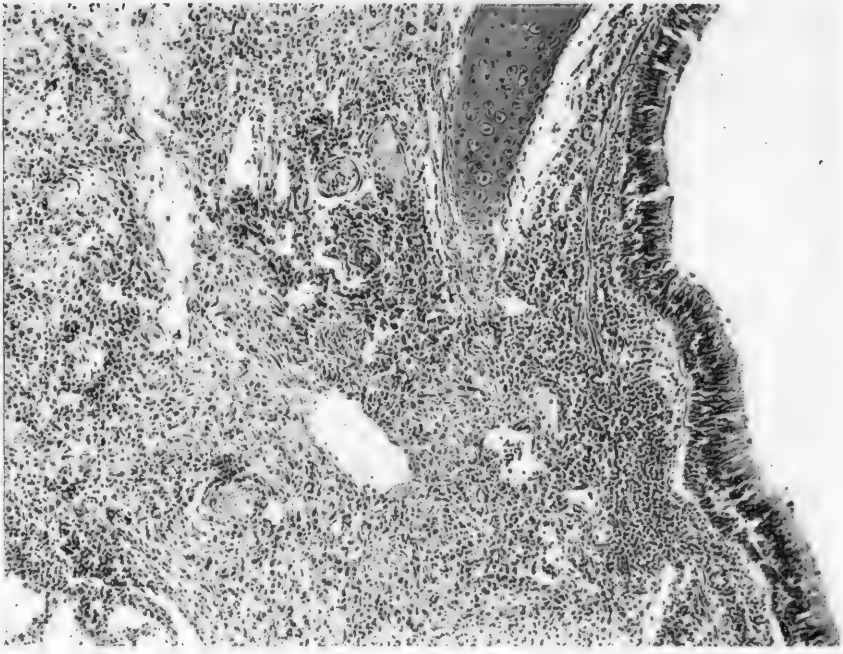


FIG. 17.

(Blake and Cecil: Experimental pneumonia. VIII.)

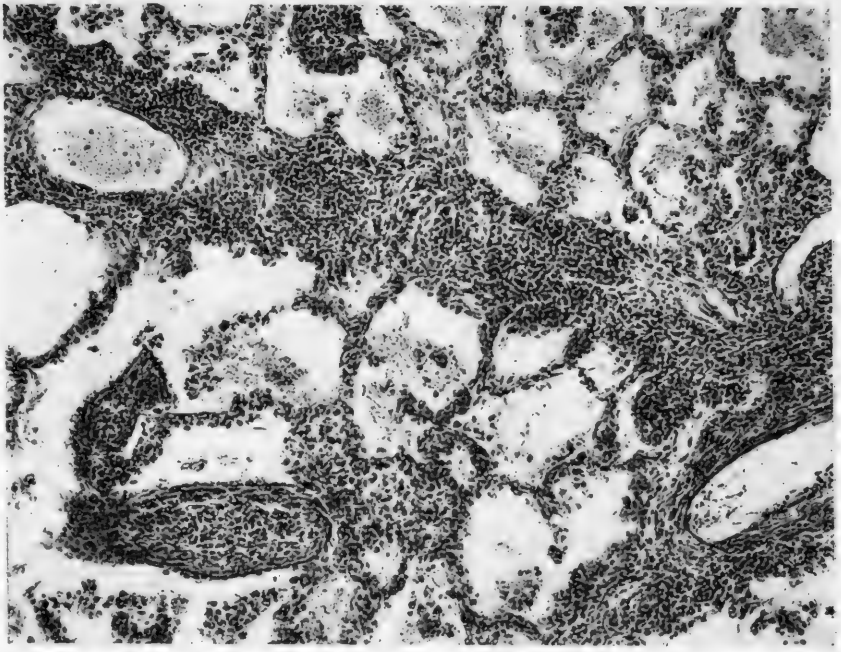


FIG. 18.

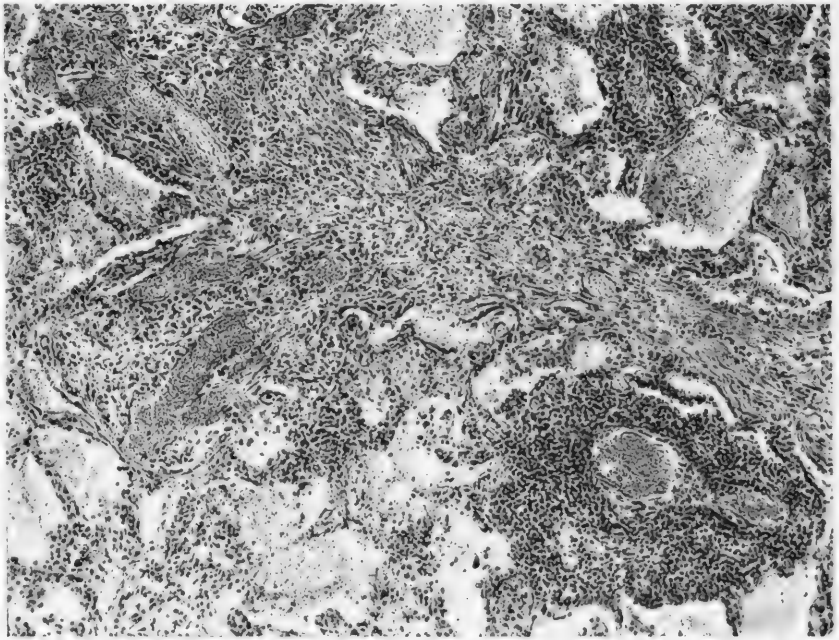


FIG. 19.

(Blake and Cecil: Experimental pneumonia. VIII.)

LOCAL AUTOINOCULATION OF THE SENSITIZED ORGANISM WITH FOREIGN PROTEIN AS A CAUSE OF ABNORMAL REACTIONS.*

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PLATES 45 AND 46.

(Received for publication, June 9, 1920.)

INTRODUCTION.

While testing the sensitiveness of a number of dogs which had been treated with horse serum some years previously, and employing heavy doses of horse serum for the reinjections, it was observed that a peculiar edema developed at the site of the operation wound in the inguinal region. This edema was noted about 2 days after the test and formed a fairly extensive, thick, brawny mass of tissue; there was no discharge from the wound. In order to explain this peculiar type of edema, I assumed that the local reaction was anaphylactic and was produced in the following fashion. In these dogs a foreign protein (horse serum) was circulating, due to the reinjection, and therefore a certain amount of this protein ought to pass into the tissues adjoining the wound during the development of the ordinary wound edema which always follows an operation. Moreover, the amount of foreign protein acting locally would be increased by the oozing of blood, serum, plasma, and lymph into the wound from the severed blood and lymphatic capillary channels, all of which contain the antigen. As the dogs were sensitized to this foreign protein, the skin and adjoining tissues would also be sensitized and could respond by an anaphylactic reaction to this local autoinoculation of the horse serum.

Since this working hypothesis of local autoinoculation could readily be utilized to explain functional changes in any tissue capable of react-

* A preliminary note appeared in the *Proc. Soc. Exp. Biol. and Med.*, 1919–20, xvii, 93.

ing anaphylactically, and since its validity could easily be tested experimentally, and since in addition the conception was new, as far as I was aware, a number of series of experiments were carried out to determine its viability.

EXPERIMENTAL.

Method.

The first tests were made in dogs which were sensitized by the subcutaneous injection of horse serum and then reinjected intravenously after an interval of 4 weeks. At the time of reinjection a deep incision through the skin was made under ether anesthesia with antiseptic precautions, then sutured, bandaged, and the process of healing observed. Although the results were encouraging, it early became evident that practical considerations made the dog an unsuitable test object. For this reason the rabbit was finally selected, and after preliminary tests the skin was again chosen as indicator organ of the reaction. The specific site was the ear, because this could be examined with the maximum of ease and the minimum of discomfort for the animal.

The rabbits were sensitized by four injections of 4 cc. of horse serum at 4 to 5 day intervals. Two injections were given into the erector spinæ muscles and two into the peritoneal cavity. After an incubation period of 15 to 21 days they were reinjected with 10 cc. of horse serum, usually intraperitoneally. In one series half the reinjection dose was given into the erector spinæ muscles and half into the peritoneal cavity. The intravenous route for reinjection was not employed because a test showed a large number of fatalities in the sensitized group. Thus in Series 2 four out of six sensitized rabbits died in 2 to 15 minutes after the intravenous injection of 5 cc. of horse serum, which made this series valueless.

The initial local damage to provoke a local edema and inflammation was produced by painting the external surface of the ear with 1 cc. of xylol and rubbing it gently for 15, 20, or 30 seconds, the time interval depending upon the series. This skin irritant causes a marked temporary flushing of the ear vessels which is followed by a mild inflammation and edema of varying degree. It will be noticed that this skin irritant was used to bring about the same condition as the skin incision in dogs, the production of a certain degree of inflammation and exudate into the tissues. In Series 1 a chemically pure *o*-xylol (Kahlbaum)[1] was used; in the others a commercial xylol (Merck) was employed.

Commercial xylol was chosen for the later series because the chemically pure isomers were available only in small amounts and also because the commercial

[1] I am indebted to Dr. W. A. Jacobs for supplying me with some of Kahlbaum's *o*-, *m*-, and *p*-xylol. It may be mentioned that *o*-xylol is more effective in producing edema than *m*- or *p*-xylol.

product seemed to be more effective in producing a local edema. Since the commercial product varies in its percentage composition of *o*-, *m*-, and *p*-xylol, and since it probably also contains various impurities, commercial xylol (Merck) from the same container was used throughout the subsequent series.

The same dose of 1 cc. of xylol was given to all rabbits and was always measured by the same pipette.

The xylol was usually applied to the skin 30 to 45 minutes after the reinjection of horse serum; in one series the interval was 3 hours. These intervals were allowed to pass in order to permit the absorption of a certain amount of horse serum, so that any edema developing after the xylol application would contain some of it.

The rubbing of the ear was always gentle and was done with a finger covered by a rubber cot. As the ear had to be held during the rubbing, some of the xylol always moistened the finger on the inner surface of the ear. To a certain extent, therefore, xylol was applied to the outer and inner ear surfaces, though by far the most was received by the external surface. The time during which the ear was rubbed was always controlled by a clock (15, 20, or 30 seconds, depending upon the series).

For each sensitized and reinjected rabbit at least two different control experiments were carried out at the same time. In one series of controls the normal rabbits received 10 cc. of horse serum intraperitoneally 30 to 45 minutes before treating the ear with xylol (serum controls). In the second series of controls no horse serum was administered, and the only interference was painting the ear with xylol (ordinary controls). In a third group of controls the rabbits were sensitized with horse serum, and after the incubation period they were not reinjected, but xylol was applied to the ear (sensitized controls). In general each series of experiments consisted of 18 rabbits: 6 sensitized and reinjected; 6 horse serum controls; and 6 normal controls.

The horse serum[2] employed was always at least 4 months old. In the earlier series the serum was sterile and without any preservative; in later series the serum contained chloroform. When chloroform was present this was driven off by floating a sterile evaporating dish containing the serum in a dish of warm water. As far as possible the same lot of serum was used for sensitizing and reinjection.

The injections were always made with a sterile syringe, and the site of puncture was prepared by cutting the hair and treating the area with dilute tincture of iodine.

All the rabbits were examined first at 2 to 4 hour intervals; then daily; later at 2 to 3 day intervals. The entire period of examination lasted about 2 weeks when no reaction was obtained; if a reaction took place the examinations were usually continued for 3 or 4 weeks.

Most of the rabbits employed were males; when females had to be used the same number of females was added to each group in a series. All were kept in

[2] The horse serum was obtained from the New York City Board of Health through the kindness of Dr. W. H. Park, Dr. E. J. Banzhaf, and Dr. I. Greenwald.

individual cages. The weight fluctuated between 1,400 and 2,000 gm. The diet consisted of oats, hay, cabbage, and turnips; occasionally carrots were available. The ordinary gray variety formed the majority; no white rabbits were used because they could not be obtained in sufficient numbers. As far as possible young adult animals were employed; very young rabbits were never used.

Results.

Before describing the experimental results more fully, it may be stated at once that the work strongly supports the working hypothesis upon which the investigation is based. Thus in three series of experiments, totalling 53 animals, the xylol-treated ears of 36 controls showed no dermatitis with blisters and crust formation; nor were hemorrhages and gangrene observed except in one and two instances respectively. In the latter instances the loss of substance did not exceed 0.5 mm. of the ear tip. The ears of these controls were practically normal 3 to 4 days after the xylol application.

The picture was, however, quite different in the sensitized and reinjected groups. Out of seventeen rabbits, ten developed an exfoliative dermatitis a few days after the xylol treatment. The blisters and crusts covered one-third to one-half of the ear surfaces, involved the deeper tissues, and always led to dry gangrene in these particular animals. The gangrene caused a loss of ear substance at the tip, varying from 1 to 3 cm. Healing was slow, but usually complete in 3 to 4 weeks. The ear stumps were at first bald, with a glistening, thin skin; later a new growth of white hair appeared (Figs. 1 to 3).

The ear changes may now be considered more in detail. When 1 cc. of *o*-xylol or commercial xylol is applied to the hairy surface of the rabbit's ear and then gently rubbed for 15 or 30 seconds, a marked dilatation of the blood vessels is usually produced fairly promptly. This flushing of the ear lasts longer than 10 minutes, but has usually disappeared after 45 minutes. At this time the ear generally presents a faint pink flush and the vessels are narrow. After 3 to 6 hours a fair to moderate edema appears in a majority, but not in all of the treated ears in the two control groups. Within 48 hours the edema in these two groups has practically disappeared in most animals, and in 3 days the treated ears are largely normal, if we exclude a slight desquamation, a slow loss of hair which is promptly replaced by normally pig-

mented hair, and a tendency of the ear arteries to flush more readily and more strongly than in the untreated ear (Table I).

TABLE I.

Onset and Course of Edema in Series 1, 3, and 4.

Time.	Sensitized and reinjected rabbits (17).	Horse serum controls (18 rabbits).	Ordinary controls (18 rabbits).
hrs.			
3–6	No or slight edema, 14. Slight to fair edema, 3.	No or slight edema, 5. Fair to moderate edema, 13.	No or slight edema, 7. Fair to moderate edema, 11.
22–27	No or slight edema, 10. Fair to moderate edema, 1. Moderate to marked edema, 6.	No or slight edema, 11. Fair to moderate edema, 6 (1 died).	Slight edema, 16. Moderate " 1. Marked " 1.
48	No or slight edema, 11. Fair to moderate edema, 3. Marked edema, 3.	No or slight edema, 14. Fair edema, 2. Moderate edema, 1.	No or slight edema, 16. Fair edema, 1. Moderate edema, 1.
72	No or slight edema, 11. Moderate edema, 2. Marked edema, 3 (1 died).	No or slight edema, 16. Slight to fair edema, 1.	No or slight edema, 16. Fair edema, 2.
120	No or slight edema, 13. Marked edema, 3.	No or slight edema, 14. Marked edema,* 3.	No or slight edema, 18.

* This recurrent edema practically disappeared the next day and did not reoccur. See text for details.

When the xylol edema has disappeared there is usually no recurrence of it. Only three exceptions were noted in the large number of controls, and they occurred in the horse serum control group of Series 1. These three rabbits had received horse serum for the first time about 45 minutes before the ear had been treated with *o*-xylol (Kahlbaum). A fair edema developed and disappeared after 48 hours. On the 3rd day none of them showed any edema (Table I). On the 5th day (no observation made on the 4th) all three showed a strong edema which was more marked than that seen previously. The next day (6th), however, the edema had practically disappeared in all, and no further edema was noted during the next 9 days, when observations were discontinued. No exudate ac-

companied this recurrent edema, nor did any gangrene take place. One of these rabbits also showed numerous small discrete hemorrhages scattered on the central portion of the upper dorsal half of the ear.

No blisters or crusts were observed in the great majority of both control groups. In the large number of controls only two cases were seen. These two animals belonged to the ordinary controls of Group 3 and had therefore never been subjected to any serum action. The exudate which they showed was very slight, occupied the extreme tip of the ear, and the subsequent dry gangrene of the tip was less than 1 mm. (Table II). The bald tip of these ears later grew a small tuft of unpigmented, white hair.

TABLE II.

Dry Gangrene of the Ear Tip.

Series No.	Sensitized and reinjected rabbits.	Horse serum controls.	Ordinary controls.
Series 1 (*o*-xylol).	Gangrene, 4.* No gangrene, 2.	Gangrene, 0. No gangrene, 6.	Gangrene, 0. No gangrene, 6.
Series 3† (commercial xylol).	Gangrene, 3. No gangrene, 2.	Gangrene, 0. No gangrene, 6.	Gangrene, 2.‡ No gangrene, 4.
Series 4 (commercial xylol).	Gangrene, 3. No gangrene, 3.	Gangrene, 0. No gangrene, 5 (1 died).	Gangrene, 0. No gangrene, 6.

* The loss of substance in all three groups varied from 10 to 30 mm.

† Series 2 was lost through death of most of the sensitized group due to intravenous reinjection.

‡ The loss of substance was less than 1 mm. of the ear tip.

In the sensitized and reinjected series of rabbits the application of xylol to the ear produces in general the same immediate effects as those noted for the control group, except that the flushing of the ear vessels at times is not quite so prompt in the animals tested 30 to 45 minutes after the horse serum injection; in Series 5, however, in which 3 hours passed before the xylol was applied to the ear, all ten rabbits showed a prompt and strong initial vasodilatation. A similar prompt initial flushing after xylol was also obtained in the group of four

animals which were sensitized but not reinjected. The development of the pink diffuse blush which preceded the appearance of a definite edema was delayed in the reinjected series as well as in the horse serum controls of Series 3 and 4; in Series 1, however, all the rabbits, both the reinjected as well as the two control groups, showed a diffuse pinkness $2\frac{1}{2}$ hours after the xylol application.

A difference between the reinjected groups and the control groups of all the series was shown in the development, degree, and persistence of the edema called forth by the xylol. Table I summarizes these variations. From this table it will be seen that the edema in some rabbits of the sensitized reinjected groups developed more slowly, persisted longer, and reached a greater degree than in either of the control groups. The table also indicates that a somewhat larger percentage of the sensitized reinjected group apparently never passed beyond a slight degree of edema than in either of the control groups.

The most striking difference, however, between the sensitized reinjected groups and the control groups was exhibited in the development of blisters, crusts, and dry gangrene in the xylol-treated ears (Figs. 1, 2, and 3). These lesions occurred practically only in the sensitized reinjected groups, and in them ten out of seventeen rabbits showed extensive lesions. In the control groups, on the other hand, only two rabbits out of thirty-five (serum controls + ordinary controls) showed a slight crust formation at the ear tip which was followed by a minimal loss of tissue (Table II).

The blisters usually were observed in 24 to 48 hours; in one instance only was a bleb seen 6 hours after the xylol application. In typical cases the blisters appear first on the internal surface of the ear along the medial or lateral border. On the external surface usually a dry brownish black crust is first seen. The blisters appear in crops, varying in size from 2 to 4 mm. in diameter; in one instance a single large, thick walled blister 20 by 12 mm. occurred at the tip of an ear. The walls of the blisters are usually fairly thin and the blisters are at first filled with a yellowish fluid which later turns brown and forms a brownish black crust on drying. Some of the blisters also contained blood. In some instances no blisters were seen but exudate and soft crusts were the first alteration noticed. Thus extensive soft crusts were

occasionally seen for the first time 5 days after the xylol application. These crusts may be extensive and approximately one-half of the ear may be covered with them. Along the borders of the ear the crusts always extend farther towards the root than along the middle of the ear. Removal of some crusts showed that the entire skin was involved, for a raw bleeding surface was left exposed (Fig. 2).

Within 3 to 5 days the upper portion of the ear began to fold and curl slightly, and in 7 to 10 days the tip of the ear was black, dry, and hard. Separation of the gangrenous portion took place slowly and usually occurred 11 to 14 days after the xylol application, at which time the process was hastened mechanically. The loss of substance varied in the three series under discussion from 10 to 30 mm. of the tip of the ear (Figs. 1, 2, and 3). The loss of substance along the lateral border was well shown by the fact that the marginal ear vein formed part of the gangrenous area or now appeared within a millimeter or less of the edge of the ear for a portion of its course.

Removal of the dry gangrenous tip and crusts left the upper ear practically bald; the skin was thin and glistening with a few raw and slightly bleeding surfaces; the borders were thickened, nodular, with some tongue-like projections. The entire upper ear was moderately edematous, of a diffuse bluish pink color, and the vessels were moderately blurred on transillumination, probably due to the edema of the surrounding tissues. Healing was usually complete in 3 to 4 weeks.

After 2 weeks the surface of the ear stump gradually became covered with hair, but the new growth of hair was entirely devoid of pigment. This white hair has now persisted without any change for more than 5 months in two typical cases which were allowed to survive.

No effort was made to follow the histological changes of the ear lesions at various stages of their development because this would have entailed the loss of too many animals and at present it was considered more important to observe the gross changes.

One more difference remains to be noted, the occurrence of numerous small discrete hemorrhages on the dorsal surface of the xylol-treated ears of sensitized reinjected rabbits. The fact that the presence of numerous small hemorrhages is being considered should be emphasized at once, for the appearance of an occasional small hemorrhagic spot or two is not infrequent in the ears of apparently normal rabbits. The petechial hemorrhages under discussion were most frequent

in the sensitized reinjected rabbits of Series 1 in which four out of six rabbits exhibited them. Among the twelve controls of this series only one animal, a serum control, developed similar hemorrhages. In Series 3 only one animal showed these hemorrhages, and it was a member of the sensitized reinjected group; the twelve controls showed no hemorrhages. In Series 4 only two rabbits developed petechiæ of the ear, and they again belonged to the sensitized reinjected group; in this series also the twelve controls showed no hemorrhages. To sum up, therefore, petechial hemorrhages occurred in seven out of seventeen sensitized and reinjected rabbits, but only once in thirty-six controls; they were especially frequent in Series 1 (four out of six) and infrequent in Series 3 and 4 (one or two out of six). This difference in frequency of occurrence in the three series is possibly related to the fact that in Series 1 chemically pure *o*-xylol (Kahlbaum) was employed, while in Series 3 and 4 commercial xylol (Merck) was used.

Supplementary Experiments.

Three other series of experiments were carried out for reasons which will be considered later. In the first of these, Series 5, the effect of allowing 3 hours to elapse between the injection of horse serum and the application of xylol was investigated. This series was composed of ten rabbits all of which had been sensitized, but not to the same degree. The number of sensitizing doses given were as follows: 6 injections, 2 rabbits; 2 injections, 3 rabbits; and 1 injection, 5 rabbits. All these animals had been used previously; two had been members of a sensitized reinjected group and the other eight had served as serum controls. 21 days had passed since the last serum injection and the last xylol application. On the day of the test each rabbit received 10 cc. of horse serum intraperitoneally; after 3 hours, none of them having shown any signs of collapse, the left ear was treated with 1 cc. of commercial xylol and gently rubbed for 15 seconds. It should be added that the left ear had not been subjected to the action of xylol in previous tests.

Since these animals form three groups of varying degree of sensitization, it is perhaps of little value to compare them in detail with the series previously described, which again have another degree of sensitiveness. The main point is that one rabbit in each set, that is, three rabbits in all, developed a marked exudate and crust formation on the xylol-treated ear. These crusts were first noted in one rabbit on the 4th day and in the other two rabbits on the 7th day (no obser-

vations unfortunately were made between the 4th and 7th days). The crusts involved both ear surfaces, varied in extent from 2 to 5 cm., and were in all respects apparently similar to those observed in Series 1, 3, and 4. The resultant gangrene, however, was much less than that observed in the earlier experiments; one rabbit lost 6 mm. of the ear tip, and the other two suffered but a slight loss of ear tissue. In the last two cases the bald ear tip was bluish, puckered, and considerably thickened, in one instance to 3 mm. About 3 weeks after the application of the xylol, and 2 weeks after the removal of the crusts, the bald ear tips showed a new growth of white hair.

For the sake of completeness it may be added that after 6 hours the edema produced by the xylol application was only slight in seven rabbits and between slight and fair in three. In the latter three rabbits, the ear later developed blisters, exudate, and crusts as described above. After 48 hours the edema had disappeared in practically all. These rabbits, therefore, as far as delayed onset of the edema and development of a severe dermatitis were concerned, resemble the sensitized reinjected groups of previous series; they differ, however, from the latter in the relatively short duration of the edema which was produced.

The same animals, with the exception of one which was killed, were again used 22 days later in order to test the effect of xylol on the ears of sensitized but not reinjected rabbits. The rabbits therefore were only subjected to the action of 1 cc. of commercial xylol applied to the same ear which had served in the previous test; the ear was then gently rubbed for 15 seconds. An edema was noticed after 2 hours which increased moderately after 6 hours and practically disappeared after 24 hours. No blisters, crusts, hemorrhages, or gangrene of any degree were observed during the next 13 days, at the end of which time the animals were discarded. It will be observed that this group of sensitized but not reinjected rabbits behaved like ordinary normal control rabbits.

In order to verify still further the action of xylol upon sensitized but not reinjected rabbits, five normal animals were each sensitized by four subcutaneous injections of 1 cc. of horse serum, at 3 to 6 day intervals. When 21 days had elapsed after the last serum injection the left ears of the four survivors[3] were treated with 1 cc. of com-

[3] One rabbit died during the process of sensitization.

mercial xylol, and then lightly rubbed for 15 seconds. All the ears flushed promptly and strongly on application of the xylol; after $1\frac{3}{4}$ hours all showed a fair to moderate edema which increased up to 6 hours after the xylol treatment. 22 hours afterwards the edema in all had decreased, and in 48 hours it had practically disappeared. These rabbits were examined daily for 14 days and at no time were any hemorrhages, blisters, or crusts seen. In this group again the sensitized but not reinjected rabbits had behaved like the ordinary control groups.

DISCUSSION.

It has been clearly demonstrated above that a mild skin irritant, such as xylol, produces strikingly different effects when applied to the ears of control rabbits or to the ears of rabbits previously sensitized and reinjected with horse serum. In normal as well as in serum control animals or rabbits which have been sensitized but not reinjected, the xylol produces only a moderate inflammation with edema, and no blistering or obvious gangrene results. In sensitized and reinjected rabbits, on the other hand, xylol exhibits in a majority of the rabbits a marked blistering effect, followed by heavy crust formation and tissue destruction; the tissue destruction often leads to a dry gangrene involving several centimeters of the ear tip. It is thus seen that xylol acts like a rubefacient in the three types of controls, while in the sensitized and reinjected rabbits the same agent, applied in the same dose and in the same way, behaves like a vesicant and escharotic.

In an interpretation of these findings the most obvious, indeed the only explanation is that an anaphylactic reaction plays a dominant part, since the lesions were observed in sensitized and reinjected rabbits only. The ear lesions, therefore, may be the direct expression of a local anaphylactic reaction, as the working hypothesis postulates, or they may be the secondary result of an anaphylactic reaction or reactions taking place elsewhere than in the ears.

In an analysis of the view that the lesions are the secondary result of anaphylactic reactions the general reactions which come into consideration are (1) an anaphylactic fall of general blood pressure; (2) local changes in the ear circulation due to general anaphylactic reaction; (3) anaphylactic abnormalities of cardiac action; and (4) anaphylactic changes in the blood.

Fall of Blood Pressure.[4]—Though no blood pressure determinations were made for obvious reasons, it can be definitely stated that a profound drop did not occur. This is demonstrated by the fact that none of the sensitized rabbits showed a definite prostration or collapse after the reinjection, a condition which always accompanies a severe lowering of the blood pressure in anaphylactic rabbits. If a fall of blood pressure occurred, it therefore must have been at most a moderate drop. Concerning the duration of this hypothetical blood pressure fall, indirect evidence must be sought from experiments in which the reinjected dose was given intravenously, for there is no record of blood pressure studies in the anaphylactic rabbit when the reinjection is carried out by way of the peritoneal cavity, which is the route employed in the experiments reported in this paper. Now it may be justly assumed that the blood pressure fall of sensitized rabbits is surely not severer or of longer duration in rabbits reinjected peritoneally than in similarly sensitized rabbits in which the intoxicating dose is administered intravenously. I have numerous instances of the latter type from earlier experiments,

TABLE III.

Blood Pressure Fall of Sensitized Rabbits Reinjected Intravenously.

	Normal blood pressure.	Lowest level after injection.	Recovery level.	Time required for recovery level.	Dose of serum.
	mm.	*mm.*	*mm.*	*min.*	*cc.*
1	110	56	103	20	10
2	104	63	103	30	—
3	113	80	110	12	10
4	116	51	108	25	10
5	130	85	108	17	20
6	120	79	110	6	10

and Table III illustrates the degree of blood pressure fall and the time required to regain a practically normal level. These rabbits were sensitized by repeated (spaced) injections of horse serum at intervals, and were reinjected intravenously with 10 cc. of the same serum. The blood pressure was recorded with a Hürthle membrane manometer. Many mates of these animals succumbed acutely to the same reinjection dose.

Table III illustrates how promptly, within 6 to 30 minutes, well sensitized rabbits may regain a practically normal blood pressure level even though the anaphylactic blood pressure drop is severe.

It must be remembered that the xylol was never applied to an ear until at least 30 to 45 minutes had elapsed after the intraperitoneal injection of 10 cc. of horse serum; in one series (No. 5) 3 hours were allowed to pass. It may then be concluded with a fair degree of certainty that all the animals had recovered

[4] Arthus, M., *Arch. internat. physiol.*, 1908–09, vii, 479.

largely if not entirely from the anaphylactic fall of blood pressure before the xylol was applied to the ear. The anaphylactic fall of blood pressure, therefore, may be disregarded as a factor in the production of the ear lesions.

Local Changes in the Ear Circulation.—A factor of perhaps greater importance than a fall in the general blood pressure is the degree of circulation which is maintained in the xylol-treated ear. The circulation in the treated ears of the sensitized reinjected rabbits might be less efficient than in the xylol-treated ears of controls. This factor was controlled as far as possible by inspection at 2½, 3, and 6 hour intervals after the application of the irritant. There was no significant change in the vascularity of a majority of the ears in the sensitized reinjected groups which was not also present in a majority of one or both control groups at the same time. One factor perhaps indicates that the circulation in the xylol-treated ears of the sensitized reinjected group was not so good as in the control ears; *viz.*, the fact that the edema developed more slowly than in the control group. It is also possible, however, that this delay in the onset of edema is the result of blood changes which will be touched upon later. On the whole, however, there is no definite evidence that the circulation is poorer in the sensitized reinjected group of rabbits after the application of xylol than in the control ear.

Abnormalities of Cardiac Action.[5]—These are very frequent in anaphylactic rabbits after intravenous injections but their duration is relatively short. As a rule they last only 7 to 21 minutes after the reinjection. Consequently, even if it is granted that the same cardiac changes appear in sensitized animals after intraperitoneal reinjection, the animals had ample time to recover before the xylol test was made (30 to 45 minutes and 3 hours after the serum injection).

Anaphylactic Changes in the Blood.[6]—What effect variations in the coagulability of the blood, alterations in the blood picture, and other anaphylactic hemic changes have upon the resistance of body cells is not known, and their influence in the production of the xylol ear lesions cannot therefore be estimated. As mentioned before, these factors may perhaps be the cause of the delayed onset of the edema in the sensitized reinjected animals.

An observation which deserves mention is that the xylol-treated ears of the sensitized and reinjected group often remained cooler than the non-treated ear of the same animal for some hours; after this period they became warmer than the non-treated ear. The treated ears of the control groups, on the other hand, usually were warmer than the non-treated ears within 2 hours after the application of the xylol. There was, however, no relation between the incidence of blisters, skin destruction, and gangrene and this initial coolness of the ear; the lesions

[5] Auer, J., and Robinson, G. C., *J. Exp. Med.*, 1913, xviii, 450.

[6] Arthus, M., *Arch. internat. physiol.*, 1910, ix, 157. von Pirquet, C., and Schick, B., Die Serumkrankheit, Leipsic and Vienna, 1905. Schlecht, H., and Schwenker, G., *Deutsch. Arch. klin. Med.*, 1912, cviii, 405. Weinberg, M., and Séguin, P., *Compt. rend. Soc. biol.*, 1914, lxxvi, 585.

occurred and failed to occur in ears which had been cooler than the untreated ears for the first few hours; similarly they appeared or failed to appear also on ears which had been warmer than the untreated ear of the same animal. There was apparently, however, a definite relation between the initial coolness of the treated ear in the sensitized reinjected group and the subsequent degree of edema; if the ear was initially cool for some time, the edema which developed later (before the onset of skin inflammation) was generally slight. From the facts stated above it follows that the degree of edema bears no relation to the incidence of ear lesions. Typical lesions of the treated ear in the sensitized reinjected groups occurred after slight, as well as after marked edema.

From this survey it may be concluded that none of the factors discussed offers a well founded, satisfactory explanation of the ear lesions; in spite of this, however, it is possible that some, or perhaps even all may play a part in the circle of events which are necessary to produce the end-result.

We come now to the alternative explanation, that the ear lesions are the expression of a primary, local, anaphylactic reaction. This interpretation is the one upon which the investigation was founded and which was briefly stated in the beginning of this paper. The working hypothesis assumed that a sensitized organism, under certain conditions, could reinject itself locally with the same antigen which caused sensitization, thus producing the state necessary for an anaphylactic reaction in that locality. The conditions were (*a*) the presence of the antigen in the circulating juices of the sensitized organism, and (*b*) the local accumulation of this antigen in a tissue showing some degree of inflammation.

These conditions were met experimentally by employing rabbits sensitized with horse serum, and, after an incubation period, reinjecting them intraperitoneally with a readily tolerated dose of the same serum. After a time the blood and lymph would necessarily contain some of the horse serum due to absorption from the injection depot. The local accumulation of the antigen in an inflamed area (condition *b*) was brought about by the application of a mild skin irritant, xylol, upon the ears of the rabbits. As this irritant causes inflammation and edema of the ear tissues, it is obvious that the edema fluid would contain more or less of the horse serum, for the material composing the edema must necessarily come from the blood and lymph capillaries in which this antigen is circulating and whose permeability has

been increased by the inflammatory process. A reaction between the sensitized inflamed tissue cells and the antigen could now take place even though the concentration of the horse serum were not greater in the inflamed and edematous area than in the lymph spaces of non-inflamed tissues of the same animal. The reason is that in the inflamed tissues the metabolism of the cells is greatly increased, and we may therefore justly assume that a larger amount of antigen-bearing material affects the inflamed sensitized cell per unit of time than affects the non-inflamed cell in the same interval. Thus the stimulus of a certain concentration of horse serum which is subliminal for a non-inflamed sensitized cell, may rise above the threshold value when an inflamed sensitized tissue is concerned, and an anaphylactic reaction occurs.

On the basis of this interplay of known conditions, the lesions observed in the sensitized and reinjected rabbits are easily explained as the expression of a primary, local, anaphylactic reaction in the skin and subcutaneous tissues of the xylol-treated ears.

Since the explanation and working hypothesis demand the interaction of antigen and sensitized cells of the skin, the phenomenon of Arthus[7] suggests itself at once as a well known example of this type of reaction, and I believe that the ear lesions described above are a variation of Arthus' phenomenon. There are, however, fundamental differences between the procedure resulting in the Arthus reaction and that causing the ear lesions described in this paper.

The Arthus reaction is a skin reaction only, and results when a repeatedly injected rabbit receives a final dose of antigen subcutaneously, the site of the last injection then developing the lesion, the severity of which depends upon the degree of sensitization. In the procedure described in this paper the sensitized animal reinjects itself where there are inflammation and edema, provided that some of the antigen is circulating, the amount circulating being so dilute that it is ineffective under ordinary conditions. Another fundamental

[7] Arthus, M., *Arch. internat. physiol.*, 1908–09, vii, 471. In this article the three notes originally published in the *Compt. rend. Soc. biol.*, 1903 and 1906 (Arthus, M., *Compt. rend. Soc. biol.*, 1903, lv, 817; Arthus, M., and Breton, M., *Compt. rend. Soc. biol.*, 1903, lv, 1478; Arthus, M., *Compt. rend. Soc. biol.*, 1906, lx, 1143), are reprinted.

difference is that the site of reaction, according to this conception, is not always the skin, as in Arthus' phenomenon, but may be any tissue capable of exhibiting an anaphylactic reaction. The reaction, therefore, may take place in the skin, the bronchial muscles, the gastrointestinal canal, the heart, the pulmonary artery, and the nerves, which are structures in which anaphylactic reactions have been described.

That the skin was chosen in the present research was a mere matter of convenience. It was selected because the changes could be directly observed at any time, thus avoiding the complication and difficulties due to indirect observation which always demands instrumental aid. As a matter of fact, preliminary series of experiments had been made on the kidney and on the intestine to test the working hypothesis, but they were abandoned because for the time being it was impossible to carry out the necessary routine work in series of animals sufficiently large to warrant the trial.

It is possible that this process of autoinoculation will aid in explaining the causation of a number of more or less temporary functional derangements of obscure origin in the human subject. In my opinion it is quite probable that sensitization to one or several alien proteins exists much more frequently in man than is suspected, the undenatured protein gaining access to the circulating juices by way of a permeable gastrointestinal mucosa or the nasal and pharyngeal mucous membranes by inhalation; the uncritical use of sera and vaccines at the present time also undoubtedly adds to the number of those sensitized unnecessarily. Severe intoxication of the sensitized individual, however, occurs but rarely since the amount of foreign protein necessary to cause obvious general symptoms on reinjection exceeds hundreds and thousands of times the amount which sensitizes. It is in such individuals that the mechanism described in this paper may come into action and produce a large variety of effects.

It is fully realized that the working hypothesis here developed at some length is not absolutely proved by the experimental data and that much more work is necessary to establish it definitely. It is believed, however, that the experimental results support the conception and that this view renders some functional abnormalities in the human subject more accessible to our understanding.

SUMMARY.

The skin irritant, xylol, when applied to the ears of sensitized and reinjected rabbits often causes a severe inflammation with the formation of crusts and destruction of tissue. Dry gangrene of the entire ear tip may result (Figs. 1, 2, and 3).

The same agent, applied in the same dosage and in the same way, to the ears of (1) normal rabbits, (2) normal rabbits injected once with horse serum (serum controls), or (3) sensitized but not reinjected rabbits, causes only a mild inflammation with more or less edema. The inflammation and edema disappear in 2 or 3 days, leaving a practically normal ear.

The ear lesions of the sensitized reinjected rabbits which develop after the application of xylol are interpreted as a primary anaphylactic reaction. This primary anaphylactic reaction is considered the result of a local autoinoculation of the ear tissues with circulating antigen. The local autoinoculation is brought about by the irritant action of the xylol which causes an inflammation and edema of the site of application. An anaphylactic reaction may now occur because the inflamed tissues are more active metabolically than normal tissues and therefore the inflamed cells are affected by more antigen per unit of time than the normal cells. A subliminal concentration of antigen for non-inflamed, sensitized cells may thus become effective when inflamed sensitized cells are concerned.

This process may theoretically occur in any tissue of a sensitized animal which can show an anaphylactic reaction, for example the intestines, lungs, heart, skin, nerves, arteries, etc. It is possible that this interplay of conditions may explain a number of functional abnormalities in the human subject.

EXPLANATION OF PLATES.

The three figures were made from a rabbit of Series 3. Sensitization, reinjection, and application of 1 cc. of commercial xylol to the left ear were carried out as described in the text.

PLATE 45.

FIG. 1. 12 days after the application of xylol; internal surface. The figure shows well the curling of the ear tip, which was dry and hard, the occurrence of hemorrhages near the gangrenous part, and the appearance of discrete blisters.

FIG. 2. 20 days after the application of xylol; dorsal surface. The dry, gangrenous tip has been removed, leaving a thickened, nodular, irregular, bluish border. Some of the crusts on the dorsal surface have also been removed to show the extensive destruction of the skin.

The dorsal surface of the right ear shows a central area where a moderate dermatitis with crust formation took place; this was due to contact of the right ear with the left shortly after application of xylol to the latter. The same effect was observed repeatedly in the sensitized reinjected groups, but not among the controls.

PLATE 46.

FIG. 3. 30 days after the application of xylol; dorsal surface. The ear has healed practically completely; note the thickened borders. The ear is largely bald but near the root some white hair is beginning to appear. This white hair gradually covered the ear stump and has remained white now for 5 months.

The right ear patch has also healed completely and shows a few white hairs. 5 months later the hair was still white.

Fig. 1.

Fig. 2.

(Auer: Local autoinoculation.)

FIG. 3.

(Auer: Local autoinoculation.)

EXPERIMENTAL SYPHILIS IN THE RABBIT.

IV. Cutaneous Syphilis.

Part 1. Affections of the Skin and Appendages.

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Plates 47 to 58.

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In the preceding papers of this series (1–4), experimental syphilis in the rabbit has been presented from the standpoint of the phenomena of the infection which develops about the site of the inoculation. Ordinarily, these are the only manifestations of infection which are observed in the rabbit, but under certain circumstances, a generalized disease may be produced which is analogous in many respects to that of man, and in taking up the generalized infection, it will be necessary to give a brief résumé of the development of this phase of the subject in order that our work may appear in the proper relation to that of other investigators.

When it had been clearly established that the syphilitic infection could be transmitted to rabbits and maintained with undiminished virulence through successive generations of transfers, interest began to be centered upon problems of a generalized infection. From the outset, opinion was divided as to whether the infection in the rabbit became generalized as in man or remained essentially a localized infection. This division of opinion was in part an outgrowth of the controversy in regard to generalized infections in the lower monkeys and in part a result of experimental observation.

At all events, the development of lesions at points remote from the site of inoculation appeared to be an exceptional occurrence. The first instance of an infection of this kind was reported by Grouven in a series of communications beginning in 1907 (5–8). This case has become classic, and it is worthy of note that it still is the most pronounced example of a generalized infection following local inoculation which has been recorded in the literature. In fact, with a few exceptions, this animal exhibited all the manifestations which have since been

recognized as characteristic of generalized syphilis in the rabbit. In brief, the conditions noted included weakness, emaciation, dyspnea, alopecia, papular and maculopapular lesions of the skin, infiltrations and rhagades about the anterior nares associated with a mucopurulent discharge, conjunctivitis, a metastatic keratitis, and an infection of the testicles, epididymis, and regional lymph nodes, all developing from a unilateral infection of the eye and subsequent enucleation of the infected organ. The first manifestations of a generalized infection appeared 8 months after inoculation and active lesions were still present when the animal died 9 months later.

These observations of Grouven stimulated a great deal of discussion concerning generalized syphilis in the rabbit. Grouven himself reported two other cases (8), and in 1909, a fourth case consisting of a double keratitis originating from a testicular inoculation was reported by Menzincescu (9). About the same time, Uhlenhuth and Mulzer (10) reported a case of metastatic involvement of the scrotum of an uninoculated testicle with papular lesions about the anus, and Truffi (11) reported two cases of keratitis in animals inoculated in the scrotum. Subsequently, other cases of generalized infection were added to this list, but the total number of cases recorded in the literature has remained comparatively small, and most of the animals have shown only a few lesions of a minor character.

In addition to occasional instances of animals showing generalized lesions, there were other facts, however, which indicated that the infecting organism itself was not confined to the region of inoculation. As has been mentioned, Ossola (12) and Truffi (11) demonstrated the presence of spirochetes in the inguinal lymph nodes of rabbits inoculated in the scrotum in 1909, and as early as 1908, Neisser (13) reported successful inoculations of monkeys with material from the spleen and bone marrow of three out of seven rabbits killed 7 to 8 weeks after inoculation with a spleen-bone marrow emulsion from monkeys. These rabbits had been inoculated in the testicles, and although no local lesions had been detected up to the time they were killed, it was claimed that the virus was recovered from the internal organs. As reported by Neisser, these experiments might not be regarded as conclusive, since the only evidence of a specific infection submitted was the production of lesions in the inoculated monkeys which were said by Siebert to be typical primary lesions. At first, other investigators had difficulty in verifying these results, but eventually Truffi (14) succeeded in one instance (number of attempts not stated) in producing a definite infection by inoculation of bone marrow from an animal infected in the scrotum, and similar results have since been obtained by other investigators, notably by Uhlenhuth and Mulzer (15). The obvious objection which might be raised to these experiments, however, is that the results obtained appear to have been inconstant, and no one could say what proportion of the animals would have developed generalized lesions had they been permitted to live.

The occasional case of an animal with manifestations of a generalized infection or the recovery of the virus from the blood and internal organs of even a small

proportion of infected animals was sufficient, however, to establish the fact that generalization of the virus did occur in some instances, but no large series of experiments was carried out, so far as we are aware, for the purpose of determining the time and frequency or the extent of the generalization which took place, and this type of information was much needed to place the question of a generalized infection upon a proper basis.

Further than this, there has been little effort to correlate the phenomena of generalization and the production of generalized lesions. All that was determined was that some animals did show a dissemination of the virus, but in the experience of most observers the appearance of lesions other than those at the site of inoculation was the exception rather than the rule. For example, out of some hundreds of rabbits studied by Uhlenhuth and Mulzer (15), there were comparatively few which showed generalized lesions other than a metastatic infection from one testicle to the other or an occasional case of keratitis, and again Baeslack in 1916 (16) reported that there were no cases of generalized lesions in about 800 rabbits which he had studied.

On the other hand, a few investigators have observed generalized infections with greater frequency. In 1914 Nichols (17) reported the occurrence of generalized lesions of various types in one-half of a series of animals inoculated with a nervous strain of *Treponema pallidum*. Among the conditions mentioned were papular lesions of the eyelids, metastatic lesions of the scrotum and testicles, and lesions of the eyes. In this last group, there was included a new form of lesion of the eye ground recognizable by ophthalmoscopic examination. Unfortunately, neither the relative nor the absolute frequency of the various types of lesions was given, but the investigations were continued by Reasoner (18) who mentions among the manifestations produced by this organism "fundus involvement in about 75 per cent of the rabbits, extension to the opposite testicle in 10 per cent of cases, keratitis occasionally, involvement of the nasal mucosa frequently, with the presence of organism in the nasal discharge. Periostitis of the nasal bones is a later manifestation. There are eyelid lesions in 5 per cent. This strain also may cause mucous lesions of the penis and sheath. It occasionally causes a paronychia, and often a moderate degree of alopecia." But here again, Reasoner frequently refers to the use of repeated and combined testicular and intravenous inoculations, and it is not clear whether the facts recorded above represented results of testicular inoculation alone or included also results obtained from mixed and repeated inoculations of various kinds. In his conclusions, he states that "ordinarily the rabbit develops only an initial lesion, following inoculation in the genitalia, eyes and eyebrows."

On the whole, therefore, our knowledge of generalized syphilis in the rabbit resulting from local inoculation has rested to a large extent upon a proven dissemination of the infecting organisms, the time, frequency, and extent of which were unknown, and the occasional development of a few lesions at some point more or less removed from the site of inoculation, but no comprehensive description has been given of the infection thus produced.

Uhlenhuth and Mulzer (19, 20) described a generalized infection in the rabbit produced by a distinctly different mode of procedure, but even here the picture presented is incomplete. Since these investigators were unable to obtain a severe generalized infection from a local inoculation, they attempted to produce such a condition by resorting to a generalized inoculation, using intracardial and intravenous injections of very large doses of organisms. In this way, they succeeded in producing with considerable frequency what were in reality multiple primary lesions, first in young rabbits and then in adult animals. Considering the mode of inoculation employed, these infections were, of course, more analogous to intrauterine than to postnatal infections. Clinically, they were characterized by emaciation and weakness, especially in young rabbits, associated with lesions of the skin and appendages, the mucous membranes, the genitalia, the eyes, and tumor-like masses about the nose and tail. The extent of the involvement as well as the number and character of the lesions present varied considerably in different animals; there was an especial tendency to exudative affections of the mucous membranes, and the nose and tail tumors together with affections of the nails were the chief additions to the list of syphilitic manifestations which had been previously described. In other words, the lesions produced in these animals were of much the same character as those which had been reported in cases resulting from local inoculation but were, on the whole, more marked and could be produced with much greater frequency.

These experiments appear to have been accepted as a basis for constructing the picture of generalized syphilis in the rabbit and lent considerable support to the contention that marked generalized infection from local inoculation did not occur except in an occasional animal. Of course, the fallacy in this line of reasoning lay in the apparent assumption that a widely disseminated primary infection such as that produced by Uhlenhuth and Mulzer was comparable to conditions which obtained in the secondary diffusion of organisms from an established focus of infection. This error of conception, as will be seen, was a very serious one and had the effect of diverting attention from fundamental problems of the infection.

Since the publication of the work of Uhlenhuth and Mulzer, interest in experimental syphilis has abated somewhat and the only contributions of note which have appeared during the past few years are those of Nichols and of Reasoner.

While these earlier observations were doubtless correct as far as they went, the impressions created were founded upon an imperfect understanding of the experimental infection; they not only left unsettled many questions relative to the generalized infection but gave an erroneous impression as to the nature of the infection produced by local inoculation.

We have been able to collect a large amount of material bearing upon this phase of experimental syphilis which will be presented in

the following series of papers dealing with clinical manifestations of generalized syphilis in the rabbit. We regret that the pathology of these conditions cannot be presented in connection with their clinical history. We have, however, an abundance of material and hope that eventually we may be able to return to this phase of the subject and supply the pathological connection.

Source and Nature of the Material Studied.

The material which forms the basis for our description of generalized syphilis in the rabbit is composed of two groups of cases. Up to September 1, 1919, we had been able to collect 126 rabbits with outspoken manifestations of generalized syphilis developing from local inoculations in the testicles or scrotum. This list of animals included only those in which visible or palpable lesions were present at points entirely removed from the site of inoculation. Thus, metastatic lesions of the testicles and scrotum were not included among the generalized affections on account of the possible confusion of true disseminated lesions with extensions or transformations of processes arising from the primary focus of infection.

This first series of animals came from stock transfers and routine experiments connected with the use of the experimental infection in the rabbit as a means of studying the action of drugs against syphilitic infections and was composed of three classes of animals: (1) those in which the infection ran an undisturbed course; (2) those in which the generalized lesions developed subsequent to castration or excision of primary lesions; and (3) those in which the lesions followed drug administration.

From observations made in the course of routine work, together with an analysis of this group of cases, it appeared that the entire problem of animal resistance to *pallidum* infections, and hence the occurrence of generalized lesions, was intimately connected with the nature and extent of the reaction which took place at the site of inoculation, and that any influence which was capable of modifying this reaction might be expected to react upon the phenomena of the infection as a whole. In particular, it appeared that any condition which tended to lessen, to restrain, to inhibit, or to suppress this reaction without exerting a comparable effect upon the organisms them-

selves might be expected to favor the production of generalized manifestations of disease.

Accordingly, a series of experiments was carried out, aimed primarily at the fundamental problem of the nature of the animal infection and the mechanism of animal resistance. Thus, it was found that such simple means as a unilateral instead of a bilateral inoculation, unilateral or bilateral castration, or the use of therapeutic agents capable of inducing resolution of the primary lesions without destroying the infecting organisms, would completely alter the character of the infection and lead to the production of generalized lesions in a very large proportion of the animals inoculated.

From these experiments, a second group of animals with generalized syphilis has been collected which places the total number of cases available for study at well over 200.

The study of these animals was chiefly clinical. In the majority of cases, the lesions were allowed to pursue an uninterrupted course; in individual instances, they were excised for histological study, and some animals were killed at various periods of the infection in order to make a more thorough examination of existing conditions. The period of observation varied very considerably. Under the circumstances of our work, it was impracticable to hold most of these rabbits for any great period of time. The length of observation in most instances varied from a few weeks to several months; some animals were held for a year to 18 months and a few for more than 2 years.

The identification of syphilitic lesions rested upon the general character and clinical course of the lesions, the demonstration of spirochetes, and histological examination. In a few instances, therapeutic tests were used in the study of conditions of doubtful or uncertain character and as confirmatory diagnostic measures.

The manifestations of disease which we were able to recognize during the life of the animal fell into the five following groups and will be considered in this order: (1) affections of the skin and appendages; (2) affections of the mucous membranes and mucocutaneous borders; (3) affections of tendons, tendon sheaths, periosteum, cartilage, and bone; (4) affections of the eyes; and (5) lymphadenitis. In addition, certain visceral lesions, notably of the heart and of the central nervous system, were discovered at autopsy. This group of conditions will be reported later.

In presenting this material, we shall confine ourselves for the present to a general presentation of the subject, since it has been found that the incidence of different groups of affections, the character of the lesions present in a given case, their time of occurrence, and the general course of the disease are all more or less influenced by the circumstances under which the generalized disease makes its appearance.

Lesions of the Skin and Appendages.

The first group of conditions to be considered are the affections of the skin and appendages including alopecia, paronychia, onychia, and lesions of the skin proper. Affections of this class occurred in a large proportion of the animals showing manifestations of generalized syphilis, and it will be necessary to divide the subject matter of these conditions into two papers the first of which will be confined to a description of the individual affections, while the second will deal with the clinical aspects of cutaneous syphilis.

Alopecia.

Local or general roughening of the coat and falling out of the hair are among the conditions most frequently mentioned as manifestations of generalized syphilis in the rabbit and are extremely common among rabbits infected with *Treponema pallidum*, but occur also from many causes in uninfected animals, the chief ones being moulting, diseases of the skin, systemic disease, and uncleanly habits. While we are reasonably certain, therefore, that a large number of our rabbits showed abnormalities of the coat referable to their syphilitic infection, we realize that it is very difficult to identify such cases with absolute certainty. In studying these affections, we first attempted to exclude the four causes mentioned, which can be done without much difficulty in all except the moulting, or shedding, of the animal. It was found that under laboratory conditions, rabbits were very irregular in this respect and might shed their coats at almost any time from early March to late November, which left only about 3 months of the year during which one was not constantly confronted by this possibility. As far as we were able to determine, there was no pathognomonic sign of syphilitic alopecia in the rabbit. Among the animals studied, there were comparatively few in which we felt justified in making such a diagnosis, and in nearly all instances the diagnosis was supported by the presence of other lesions whose character could be established with absolute certainty.

As nearly as could be determined, there appeared to be at least three conditions which might be regarded as syphilitic alopecia. The most common of these

was one in which the coat became roughened or unkempt; the hair was dry and without luster and was continually falling out. This condition might affect the entire coat, as in Fig. 1, or only some smaller area, and was especially common about the head and ears, giving to these parts a characteristic moth-eaten appearance (Fig. 2). This form of affection is probably the one usually referred to as syphilitic alopecia in the rabbit.

No true baldness was noted in affections of this kind. In exceptional instances of diffuse alopecia, there was decided thinning of the hair over certain areas of the body such as the thighs, the thorax, the abdomen, or about the elbows (Fig. 3). Facial alopecia, alopecia of the ears, and of the cheeks were more marked as a rule, and the hair frequently came away from these areas in considerable masses (Figs. 3 and 4) or with gentle rubbing patches were left which were entirely denuded of hair.

A second form of alopecia which might be referred to a syphilitic infection was characterized by no other manifestation than looseness of the hair. The coats of these animals appeared to be in perfect condition but upon gently plucking at the hair, it was found to be so loose as to come away in handfuls, leaving the skin perfectly bare or covered with a short stubby growth of hair as in Figs. 5 and 6. This condition was so marked in some animals that it was possible to pluck the fur from large areas of the body without inflicting the slightest traumatism to the skin. In some instances, the skin itself appeared entirely normal, while in others, removal of the hair revealed the presence of unsuspected lesions (Fig. 5). A similar condition was found to occur in some animals at the time of shedding. In these cases, however, the growth of new hair was nearly always well advanced before the old hair could be removed, which was not the case with the supposedly syphilitic affection.

Both these forms of alopecia were only temporary affections as a rule, and after a few weeks or months, the coat returned to a normal condition. They appeared to be intensified, however, at the shedding periods of normal rabbits, which in itself is not surprising. In one animal of our series, which was held under observation for about 18 months, there was no time at which the entire region over the hips, thighs, and loins could not be picked entirely free of hair, and as fast as the hair returned, it could be plucked out again with but the slightest tension. A similar condition existed over other portions of the body, but otherwise the coat appeared to be in remarkably good condition, as may be seen by reference to Fig. 6.

A third form of alopecia which bordered upon a true baldness was seen in a few animals. This condition was usually confined to an area a few centimeters in diameter and was characterized by what might be called a peeling of the fur, the roots of which were matted together by superficial layers of the epidermis. By rubbing these areas, masses of fur came away, leaving a bare skin covered with fine epithelial scales. The skin showed a variable degree of thickening, and in some instances focalized infiltrations and necrosis as well. A typical

though rather pronounced case of this kind is illustrated in Fig. 8. The condition here shown extended from the cheeks down over the neck and shoulders where there were two well defined areas of infiltration and necrosis of the skin.

With recovery of these lesions, there was a thin stubby growth of hair but no return to normal as in the preceding cases. This condition might be classed as a true skin lesion rather than as an alopecia, but the alteration in the skin was so variable and in some instances so slight that the alopecia appeared to be the most characteristic feature of the condition.

There was one other condition noted which may be mentioned, the etiology of which could not be determined with certainty. It affected the hair of the face and especially that over the bridge of the nose, and was characterized by a thinning of the hair over the affected area while that remaining appeared broken and irregular much as in the case of tinea infections (Fig. 7). The skin of the affected part showed a slight infiltration in some cases, but in others was thin or atrophic. The only causative factor which suggested itself besides that of a specific infection was the rubbing of the animal against the cage, and this seemed unlikely. Once the condition developed, there was little or no tendency toward a return to normal.

Onychia and Paronychia.

Onychia and paronychia have been mentioned among the manifestations of generalized syphilis in the rabbit, but in our experience, affections of the nails which could be definitely ascribed to syphilitic infection were comparatively rare—only seven cases having been recorded among 126 rabbits and only one of these could be regarded as a true onychia.

Paronychia was first recognized by a slight reddening and swelling of the skin about the base of the nails. The skin then became thickened or infiltrated and was covered with yellow or yellowish gray scales or crusts producing a condition like that shown in Figs. 9, 10, and 12. In extreme cases, the reaction about the base of the nail was much more marked and resulted in the formation of granulomatous masses which underwent secondary necrosis and ulceration with consequent disturbance of the nutrition of the nails themselves (Figs. 11 and 12).

These conditions were usually bilateral and symmetrical and affected the nails of both the front and hind feet.

Syphilitic paronychia was found to be difficult to distinguish in some instances from a non-syphilitic affection of a similar character which is comparatively common among rabbits. This fungus or parasitic disease of the nails develops sooner or later in all rabbits with fungus or parasitic infections of the skin or external ear. While

these affections occur upon the hind feet as well as the front, they usually make their appearance about the toes on the median side of the front feet and are not confined to the region of the nails but spread diffusely over the interdigital surfaces. In contrast to this, syphilitic paronychia appeared to be more common about the nails of the lateral toes and was sharply confined to the base of the nail. Again, the parasitic disease is a steadily progressive affection and never clears up spontaneously, while the syphilitic condition is variable in its course and clears up completely without any treatment. If spirochetes can be found, they are helpful in making a diagnosis, but in their absence, one has to rely upon clinical characteristics.

A true onychia, as has been mentioned, was recognized by naked eye observation in only one of our first group of rabbits. In this case, the condition was associated with a marked paronychia of some of the toes, and the alterations in the nails were so pronounced as to leave no doubt as to the etiological factor concerned (Fig. 11). A similar but less marked condition was also presented by the animal shown in Fig. 12. In other instances it was noted that the nails showed signs of wearing short or appeared roughened and broken towards their ends and tended to split and scale just as in the case of the outer and inner toes of the animal in Fig. 11. An example of nail involvement of this type with no associated paronchyia is shown in Fig. 13.

At the time these observations were made, no relation could be established between this condition and the syphilitic infection. Subsequently, however, it was learned that alterations such as these might arise from involvement of the nail bed. In the rabbit, the nail fits closely over the terminal phalanx, and periostitis with considerable destruction of these bones may take place without giving rise to any external evidence of the existence of such an infection other than an alteration of the nails of the affected toes.[1] Onychia in the rabbit appears, therefore, to be most often associated with a periostitis and is difficult of recognition by the use of ordinary means of diagnosis. If the involvement is slight, the nail may eventually recover, but if the destruction is extensive, the effect appears to be permanent or at least of long duration.

[1] These observations were made by Dr. W. H. Brown, Dr. L. Pearce, and Dr. W. D. Witherbee in the course of a series of investigations of deep seated bony changes by the use of the x-ray, the results of which will be reported later.

Cutaneous Lesions.

The cutaneous lesions formed a very large and varied group of affections. They included lesions of the macular, papular, and nodular or tubercular varieties, and while they possessed many features in common with the cutaneous lesions of man, they differed from them in so many respects that it would be difficult to attempt a complete correlation of the two classes of lesions upon the basis of the material which is at present available. The only classification of cutaneous lesions of the rabbit which seems justifiable at this time is one based upon very broad lines of differentiation such as that afforded by developmental or pathological characteristics.

As was pointed out in connection with scrotal infections, one of the most striking features of the skin reaction of the rabbit to localized infections of *Treponema pallidum* is the tendency to proliferation on the part of the fixed tissue cells and the formation of large granulomatous lesions; a second characteristic of the skin reaction is the tendency to a more or less diffuse infiltration associated with varying degrees of desquamation of surface epithelium, exfoliation, and surface erosion, or necrosis and ulceration; while a third feature of the reaction is a localized hyperemia or even hemorrhage which is associated with varying degrees of exudation expressed chiefly in the form of an edema.

These characteristics of the primary reaction in the scrotum find their counterpart in the reaction to localized infection in other skin areas and form, therefore, an acceptable basis for a consideration of the cutaneous lesions of generalized syphilis. Upon this basis, cutaneous lesions will fall into three classes, the hyperemias, the infiltrations, and the granulomata. It should be understood, however, that there is no sharp line of distinction between these three classes of conditions as there is always a tendency to a combination of the three processes, and such distinctions as can be made must be based upon the predominance of one or another of the three forms of reaction.

In taking up the discussion of cutaneous lesions, the above sequence will be reversed in order to begin with those conditions which are the most obvious; namely, the granulomata.

Cutaneous Granulomata.

The lesions classed as cutaneous granulomata (Figs. 14 to 20 and 22) included those affections which developed in consequence of a reaction which was marked by proliferation of fixed tissue cells and the formation of circumscribed elevated nodules of a fleshy character varying from a few millimeters to several centimeters in diameter. Lesions of this type were quite common. They usually occurred singly or in small groups and as a rule were few in number, but occasionally they were fairly numerous and as many as twenty-eight lesions have been counted in a single animal at one time.

The granulomata appeared either in the form of rather diffuse areas of thickening which involved a considerable depth of the skin or as sharply circumscribed and indurated nodules (Figs. 14 and 17). The more diffuse lesions and the larger nodules were usually of a rose-pink or copper color or were paler than normal and of a faint yellow color, while the small discrete nodules were generally pale and opaque or of a decided opalescent appearance. In exceptional instances, the early lesion was of a deep violet-red color or appeared almost as thickened purpuric spots in the skin. In general, the surface of the lesion was smooth and rather translucent, but during the early stages of its growth there was no marked disturbance of the hair covering its surface. These several conditions are illustrated in the accompanying photographs (Figs. 14 to 19) which show various forms and developmental stages of typical granulomatous lesions.

The evolution of the cutaneous granulomata was usually rapid and within a week or so led to the formation of large oval or irregular spherical masses showing various types of secondary alteration such as are illustrated in Figs. 14 to 23. It is important to note, however, that not all the lesions present in a given case exhibited these changes to an equal degree. As a rule, only a few of those present developed to any considerable extent, while the others underwent involution and completely disappeared. This is a phenomenon worthy of note, since it illustrates the inhibitory influence exerted by one lesion or group of lesions upon another, as was pointed out in connection with the development of multiple scrotal lesions.

The growth of granulomatous lesions took place chiefly from the deeper layers of the skin and tended to be of a concentric type so that surface alterations were usually confined to a relatively small area. The most common changes observed were those due to necrosis. In some instances, this was very superficial and produced no more than a slight exfoliation of epithelium or surface erosion; in others, it extended to a greater depth, and the skin over the center, or even a larger portion of the lesion, became converted into a dry, adherent crust, or a depressed ulcer was formed which converted the lesion into a typical chancre-like mass.

While, as a rule, the area thus involved was small, occasionally a large part of the lesion was destroyed in this manner—the necrosis keeping pace with the growth of the lesion (Figs. 18 and 19).

Another noticeable feature of this class of affections was the preservation of the hair over a large part of the lesion which was obviously due to the nature of the skin involvement. With the advent of infiltration and necrosis of the outer layers of the skin, the hair first became thin and broken and was then lost over the affected area, but, as may be seen by reference to Fig. 19, many lesions showed practically no disturbance of the hair outside the zone of necrosis. In other instances, however, there were a more extensive involvement of the outer layers of skin and an obliteration of papillæ extending well beyond the area of necrosis. In these cases, the loss of hair was more pronounced and lesions were formed which, with their smooth and infiltrated surfaces, bore an even more striking resemblance to the typical scrotal chancre (Fig. 20). Usually the obliteration of skin papillæ and the loss of hair merely preceded the appearance of other changes, but not infrequently they marked the extent of the surface alteration, and the lesions thus formed were quite analogous to unulcerated nodular chancres of the scrotum (Fig. 22).

In many ways, the cutaneous granuloma of the rabbit may be viewed as an expression of a vigorous reaction to infection analogous in all respects to that which characterizes the reaction at the primary focus of infection. Objectively, the force of this statement may be appreciated by comparing Figs. 20 and 21 and Figs. 22 and 23 which represent corresponding cutaneous and scrotal lesions of two animals photographed at the same time. The significance of the striking similarity between these two processes of reaction will be made clear when we come to consider the factors in animal resistance and the mode of expression of this resistance.

Before leaving the subject of cutaneous granulomata, reference may be made to a similar group of lesions which originated within the subcutaneous tissues and reached a considerable size before any skin involvement took place (Fig. 24). These lesions were, of course, more analogous to ordinary gummata, or the so called tertiary skin lesions of man, than to those of earlier stages of the disease, and the few cases seen in the rabbit also appeared late in the course of the infection. For example, the animal shown in Fig. 24 had a succession of cutaneous lesions, the first appearing about $3\frac{1}{2}$ months after inoculation. There were no lesions in the subcutaneous tissues, however, until 25 months after inoculation when the lesion upon the nose developed and was followed 3 months later by other lesions of a similar character.

In all granulomatous lesions, spirochetes were numerous and could be demonstrated without difficulty as long as the lesions were active.

Cutaneous Infiltrations.

The second group of cutaneous lesions to be described (Figs. 25 to 41) includes a variety of conditions ranging from small discrete papules on the one hand to large weeping or crustaceous patches on the other, the common basis of which was a cutaneous infiltration in contradistinction to the proliferative reaction which characterized the lesions of the preceding group. According to available data, these affections occurred among our first series of animals about as frequently as the granulomata, but their incidence has steadily declined with changing conditions until, under present circumstances, they might be regarded as comparatively rare affections.[2] It should be pointed out, however, that lesions of this class were often less clearly defined and much more difficult of detection than the granulomata, and hence it is not unlikely that many may be entirely overlooked.

At the time of their appearance cutaneous infiltrations may differ very slightly if at all from early granulomatous lesions. As in the case of the granulomata, two forms of lesions could be distinguished from their mode of origin, one appearing as a somewhat diffuse process and the other as a very minute and sharply circumscribed nodule, and these characteristics tended to be preserved in the fully developed lesions of this class. There were thus formed two fairly well defined groups of conditions, the one a flattened or lenticular lesion, the other a more elevated and indurated papule.

Flattened Papular Lesions.—The initial lesion of this group (Figs. 25 and 26) presented the appearance of a simple infiltration of the skin, involving the papillary layers and varying from a few millimeters to a centimeter or more in diameter. These spots were usually of a faint pink or copper color, but in exceptional instances, the discoloration of the skin was quite pronounced, approaching in intensity the violet-red color occasionally seen in early granulomatous lesions (Fig. 26). At this stage, the affected area was raised but slightly above the surrounding skin level, the elevation being greatest at the center of the lesion and diminishing towards its periphery.

[2] Changing conditions in the life history of the organisms and the particular circumstances under which generalized lesions make their appearance in a given case are undoubtedly potent factors in determining the character which these lesions assume.

Very soon the appearance of the lesions changed, the color becoming a pale yellow or gray or deepening to a copper or brown as the case might be. At the same time, the infiltration increased and the hair over the affected area was lost to a considerable extent, while the skin became smooth and glistening and of a parchment-like consistency or was covered by thin scales of a yellow or yellowish gray color (Fig. 27).

Subsequently, numerous modifications of these lesions occurred as a result of growth or extension or from secondary alterations taking place within the affected area. The characteristic mode of growth of the flattened papular lesion was a peripheral extension of the infiltration which in itself gave rise to a variety of conditions. Most often there was produced a small but fairly well defined area of infiltration analogous to some of those shown in Figs. 28 and 31. Occasionally, however, the process assumed more extensive proportions and from one or more small lesions, there developed a widespread affection such as that seen in Fig. 28. Not infrequently, the original lesions or the center of the lesion in such cases underwent resolution with the peripheral extension of the process as may be seen by an examination of Figs. 28 and 29. There was thus formed a single annular lesion, as in Fig. 29, or an affection composed of a series of lesions grouped in similar fashion about the area in which the infection first appeared (Fig. 28).

In addition to conditions such as those described, numerous modifications of these lesions occurred which were attributable to secondary alterations. Among these may be mentioned a squamous type of lesion, a suggestion of which is given by the accumulation of scales about the margins of the lesion in Fig. 29. Another condition which was of frequent occurrence in this class of affections was exfoliation of the epithelial coverings which gave rise to moist or weeping patches or to areas of infiltration covered by thick yellowish gray or yellowish brown crusts (Figs. 31 and 33). In other instances, necrosis of a more pronounced character occurred and resulted in the formation of an ulcer, as in one of the lesions in Fig. 29, or the entire area of infiltration was converted into a dry, necrotic eschar, a condition which is suggested in Fig. 8.

While in many instances the flattened papular lesions were rather small and few in number, they were occasionally quite numerous and tended to spread and to fuse with one another, as has been described, or were processes of a rather diffuse character from the beginning and covered an area several square centimeters in extent. As might be expected from the nature of the process, this particular type of lesion was especially prone to exfoliation and necrosis and the formation of weeping patches or of lesions of a crustaceous character such as that shown in Fig. 33.

As was pointed out in connection with the cutaneous granulomata, it is important to note again that many of the infiltrative processes which have been described are closely analogous to certain forms of primary scrotal lesions. In order to emphasize this point, two sets of photographs have been inserted to enable one to make the comparison between the primary and the generalized

cutaneous reactions as they appeared in the same animal (compare Figs. 29 and 30, and 31 and 32).

Raised Papular Lesions.—The raised papular lesions of the skin (Figs. 34 to 41) analogous to the miliary or follicular syphilides of man were of three varieties. The simplest of these and the one from which the others usually developed was a small shotty nodule averaging from 1 to 3 mm. in diameter and raised a millimeter or so above the surrounding skin level (Figs. 34 to 36 and 38). These lesions were very sharply demarcated and were of a grayish white or faint copper color. In most instances, they were rather dense and opaque but occasionally were of a semitranslucent or opalescent appearance. The crest of the lesion tended to be rounded or pointed, but in exceptional instances there was a slight umbilication as in Fig. 36. Some of the lesions were also surrounded by a rich vascular network as is clearly shown in this same figure (Fig. 36).

These papules rarely persisted in the condition described for any considerable period of time but tended to undergo one of two types of alteration. Many of them underwent a central or apical necrosis with the formation of a small crust or ulcer. This was particularly the case with lesions situated about the face or ears (Fig. 37). The second modification seen was of a squamous type, and this was especially common with lesions of the upper eyelids and brows (Figs. 38 to 41). In some of these, a thick layer of epithelial scales was formed upon the crest of the lesion (Fig. 40), while in others, the entire nodule was incased in a covering of horny epithelium (Fig. 39). This group of conditions presented the general appearance of small vegetations, and their prominence was due largely to the accumulated epithelial coverings which upon removal left an unexpectedly small and rather flattened body of infiltration.

Another feature of these lesions was the tendency to occur in groups such as those shown in Fig. 39. In two animals of our series, one of which is shown in Fig. 37, the papules showed a definite circinate arrangement and a very striking bilateral symmetry.

Routine examinations for spirochetes were not made upon all the lesions of this group which were encountered, since it was desired to follow the clinical course of the lesions as far as possible, and it was found that the traumatism inflicted either by aspiration with a needle and syringe or by scarification was sufficient to disturb the development of the smaller lesions and predisposed to rapid regression or healing. However, spirochetes were demonstrated in lesions of all the types described. They were most easily demonstrated in the moist or weeping patches of infiltration and in the fleshy papules but were more difficult to demonstrate in the dry necrotic lesions and the small follicular papules. When once familiar with this group of lesions,

however, the clinical history was usually sufficient in itself to enable one to make a perfectly definite diagnosis.

While, in attempting to point out the salient features of various types of cutaneous eruptions, a distinction has been drawn between cutaneous infiltrations and cutaneous granulomata, it must be said that no complete division between these two classes of affections could be made. In reality, they tended to merge one with the other, and while the great majority of the lesions seen in the rabbit could be assigned to one or the other of these two groups, there were borderline conditions which would be very difficult to classify upon this basis.

As our investigations advanced, the difficulties encountered in this respect were increased due to a change in the character of the lesions produced by the two strains of organisms employed. This was especially noticeable in a decrease in the relative incidence of lesions which have been described as infiltrations and a corresponding increase in lesions which clinically might be regarded either as small granulomata or as unusually marked processes of infiltration. Mention is made of this fact on account of the bearing which it may have upon any interpretation which may be placed upon such conditions as manifestations of a reaction to infection.

Macular Erythema, or Roseola.

A macular erythema has never been described among the cutaneous lesions of the rabbit so far as we are aware, but in the course of our own observations, a pronounced rash of this type was seen upon the ears of a number of animals with manifestations of generalized infection. As yet, no conclusive proof of the syphilitic nature of these lesions has been obtained. Thus far, we have been unable to demonstrate spirochetes in the lesions, although we have not had an opportunity of making a thorough search for them in section. The evidence which we have rests upon clinical and histological examinations.

In its typical form, the macular erythema first appeared as slightly thickened, rose-colored spots or patches situated in the deeper layers of the skin or in the subcutaneous tissues of the outer third of the ear. The color of the rash deep-

ened to a dusky red or purple, then changed to a coppery hue, and faded, leaving a faint stain in the tissues. Occasionally, there were small petechial hemorrhages associated with the lesions and a central opaque white spot or ring which persisted after the general color of the rash had faded. A typical though rather pronounced case of macular erythema is shown in Fig. 42.

As a rule, the hair was loosened over the affected area or over a much greater area than appeared to be involved, and, in a few instances, the surface of the skin was also affected, becoming roughened and covered with scales, a condition which was especially noticeable upon the inner surface of the ears where the skin is normally quite smooth. Ordinarily, no other changes were noted, but in two instances, there was a well marked infiltration of the affected areas which made its appearance just as the rash began to fade. The ear of one of these animals and the areas of infiltration are shown in Fig. 43.

In their distribution, the macular lesions usually preserved a fair degree of bilateral symmetry. Aside from the ears, the only positions in which definite purpuric spots of the character described have been seen were the trunk and the thighs of two animals.

During the early stages of the erythema, the color was diminished by pressure or by vascular constriction and increased with dilatation of the vessels, but such influence had little if any effect upon the appearance of the rash after the purpuric stage had been reached. In general, these lesions suggested a reaction similar to the early reaction described in the scrotum following inoculation with a virus emulsion.

This macular erythema was usually fleeting and rarely persisted for more than a few days, and frequently a slight roseola disappeared within 24 to 48 hours after it was first noted. In most instances, the roseola developed early in the course of the infection, but in one animal, it was observed as late as 16 months after inoculation. Another characteristic feature of these lesions was a marked tendency to recurrent periods of eruption.

After a long series of observations, it was found that a very large proportion of the animals which showed eruptions of this type belonged to one of three classes: first, those which had shown other manifestations of generalized infection, second, animals in which such lesions were still present, or third, animals which subsequently did develop lesions of a definite syphilitic character.

Histologically, three types of change were found in these lesions. In the early stages of the erythema, the vessels of the region were

dilated and the surrounding tissues were edematous. At a later period, there was stasis as well as dilatation of the vessels and slight extravasation of red blood cells along with the edema, and the endothelial cells of the vessels were swollen. These were the usual changes, but in the most marked cases, there were in addition a slight migration of leucocytes and an accumulation of round cells. These changes are precisely those which occur with the initiation of the specific reaction in the testicle and scrotum.

The chief source of difficulty in relating the macular erythema to infection with *Treponema pallidum* is the occurrence of erythemata and vasomotor disturbances of various sorts in the ears of normal rabbits. The non-specific condition usually develops from the base of the ears and radiates outward along the marginal vessels but may assume a macular form not unlike that of the early roseola described. These conditions were found to occur especially during periods of moulting, or shedding, and at such times, thickened erythematous patches may be found on any part of the body where new hair is beginning to grow. However, the purpuric type of eruption was never observed except in infected animals, although very many normal rabbits were examined with this point in view.

As the matter stands, therefore, we feel reasonably certain that a true macular lesion does occur in the rabbit, but the difficulties of making a positive diagnosis are so great that we have confined our listing of such conditions to animals which showed some other manifestation of generalized syphilis, and among these, to those animals in which the rash appeared in typical form.[3]

Lesions of Uncertain Etiology.

The cutaneous lesions thus far described include only those affections whose etiology we have been able to establish with absolute certainty or, as in the case of the macular erythema, with a strong degree of probability. This group of manifestations would hardly be complete, however, without mention of several conditions which may

[3] It is possible that therapy exercised some influence upon the occurrence of macular erythemata, since eight of the first thirteen animals in which an outspoken eruption of this character was observed, were drug-treated animals.

bear some relation to *pallidum* infections but whose etiology we have not been able to establish to our own satisfaction.

The first of these conditions is that illustrated by Fig. 44 which shows a bare area of skin in the lumbar region from which the hair has been plucked. In this area, there were two definite nodular masses, one covered by a short growth of hair, the other entirely bare and of a decided red color. A similar reddened and thickened area projects beyond the margins of the upper edge of the denuded skin area. These conditions were comparatively common and especially so at the time of moulting.

It will be noted that the peculiar condition of the skin in this animal is not unlike the early stage of a syphilitic skin lesion but analogous conditions are known to occur among uninfected rabbits. In the presence of a negative examination for spirochetes, obviously no diagnosis of the nature of such affections could be made. Granting, however, that conditions of this kind are entirely phenomena of a new growth of hair, it still appears possible that they may be influenced to some extent by the presence of a syphilitic infection. Whether such is the case cannot be said. The conditions were confined almost entirely to the face, where known skin lesions usually present a very characteristic appearance, and to the trunk, a region in which almost nothing is known of syphilitic lesions. The condition is mentioned mainly to avoid confusion between affections of proven origin and conditions of unknown etiology which may simulate them.

The second group of conditions to which we may refer concerns more especially certain of the hair follicles or possibly the sebaceous glands of the cheeks and, to a lesser extent, the neck. In a considerable number of infected rabbits, it was found that an abnormal condition of the skin developed about the roots of certain hairs, usually late in the course of the disease. The condition was characterized clinically by the formation of an exudate which glued together small tufts of hair irregularly distributed over the cheeks and sides of the neck. This material extended a millimeter or so up the shaft of the hair and resulted in the formation of dry, hard projections which were very easily palpable and were of a cream-yellow or lemon-yellow color. When the hair was clipped, the appearance presented was that shown in Fig. 45.

This condition might persist for a very long time or might clear up after only a short duration. In the process of clearing, the hair came out, and one found beneath these masses of exudate, faint circles in the skin similar to those seen in Fig. 46.

A condition which appeared to be of the same nature developed in several animals whose cheeks had been shaved as a means of investigating suspected skin lesions. One of these animals is shown in Fig. 47. The lesions formed under these conditions were minute points of elevation surmounted by a small crust. It was found that by applying pressure or tension to the skin, a small droplet of clear or slightly turbid fluid exuded from these points. This fluid contained a

large number of polynuclear and mononuclear cells, but no spirochetes could be found.

In this particular animal, it may be of interest to note the existence of another abnormality in regard to the growth of the hair of the area shown. This animal with two others was shaved several weeks before the photograph in Fig. 47 was taken. At that time, there were several small syphilitic lesions at the base of the ears. The hair of the other two animals (both with specific lesions) returned very promptly, but in this animal, there was practically no growth of hair for many weeks which in itself is indicative of disturbed nutrition of the hair, and is a condition which has been repeatedly observed in animals with marked generalized syphilis.

This group of conditions suggests the possibility of a follicular syphilide, possibly a pustular eruption, but at present, it is no more than a suggestion. Similar conditions were found in uninfected rabbits but were far less common and were usually associated with other evidences of abnormality. In infected animals, this condition showed no relation to other diseases. For example, the rabbit in Fig. 45 was in perfect condition except for the presence of two small lesions upon the hind feet. A year previous, however, it had been the subject of a most pronounced cutaneous syphilis.

We are of the opinion that some of these conditions may be directly or indirectly related to infection with *Treponema pallidum*. We have had no opportunity, however, to make a thorough investigation of them and in the absence of any definite proof of a syphilitic origin, they must be left as conditions of uncertain etiology.

SUMMARY.

From the study of a large series of rabbits with outspoken manifestations of generalized syphilis, lesions of the skin and appendages were found to constitute one of the largest and most varied groups of such affections. The conditions noted consisted of alopecias, onychia and paronychia, and lesions of the skin proper.

It was found to be a matter of some difficulty to make a positive diagnosis of syphilitic alopecia, but there were three and possibly four conditions which appeared to be attributable to such an infection. The first of these took the form of a general or local roughening of the coat with falling of the hair which produced the typical moth-eaten appearance associated with syphilitic alopecia in the human subject. A second form of alopecia was essentially an abnormal looseness of the hair which permitted large areas of the body to be completely

denuded. The third type of alopecia was associated with definite skin changes, and the hair was readily removable together with an adherent mass of epithelial scales.

Paronychia was comparatively rare but was readily recognized by a characteristic infiltration and exfoliation of the skin about the base of the nails.

The incidence of onychia is uncertain. Late in the course of the investigation it was found that alterations in the nails which were not entirely characteristic in themselves might occur in consequence of a syphilitic involvement of the nail beds which could not be detected by ordinary methods of examination. The cases which were recognized as syphilitic were those which showed an associated paronychia.

Lesions of the skin were found to be one of the most frequent manifestations of a generalized infection in the rabbit. These lesions were divided into three classes: first, granulomatous lesions, second, infiltrations, and third, erythemata.

The granulomata were lesions of a fleshy character which tended to grow to a very large size and presented all the characteristics of circumscribed primary lesions of the scrotum.

The conditions described as cutaneous infiltrations included two general types of lesions, one a flattened and rather diffuse process, the other an elevated and sharply circumscribed papule. As a class, these lesions were very prone to secondary alterations and in this way gave rise to a great variety of conditions which in general resembled the diffuse primary lesions of the scrotum and the papular lesions resulting from local dissemination.

A third type of lesion resembling the macular erythemata of man was observed in a small number of animals, and while no definite proof of the specific origin of these lesions was obtained, the evidence available was strongly suggestive.

In addition, several other cutaneous affections were noted which have not as yet been thoroughly investigated. It is suggested, however, that these processes may bear some relation to infection with *Treponema pallidum*.

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EXPLANATION OF PLATES.

All the illustrations are from unretouched photographs which, with the exception of Figs. 1 and 6, represent the objects at approximately their natural size.

FIGS. 1 to 8. Alopecias in the rabbit. Abnormalities of the coat which may be referable to a syphilitic infection. All the animals here shown had active manifestations of generalized syphilis, other than the alopecia, at the time these photographs were taken with the exception of one in Fig. 6 and in this case there was a double keratitis at the time the alopecia first appeared.

PLATE 47.

FIG. 1. An abnormal condition of the coat frequently observed in rabbits infected with *Treponema pallidum*. A diffuse alopecia.

PLATE 48.

FIG. 2. Alopecia areata, showing a slightly moth-eaten appearance of the hair about the face and ears. This condition may be simulated by ordinary processes of moulting.

FIG. 3. Diffuse alopecia, showing a marked thinning of the hair over the thigh and a slight roughening of the skin.

PLATE 49.

FIG. 4. Alopecia areata associated with desquamation of surface epithelium, a condition also seen at the time of moulting.

FIG. 5. An animal in which the hair was plucked from the region of the head and shoulders. Elsewhere the hair was firmly set. Note the cutaneous lesions on the face. An affection as pronounced as this is rarely observed in a normal animal but is comparatively common among those infected with *Treponema pallidum*.

PLATE 50.

FIG. 6. An affection of the coat characterized by abnormal looseness of the hair which persisted over a period of more than 18 months. The region over the hips, thighs, and loins of this animal were plucked clean a number of times. Note the excellent appearance of the coat of the animal.

PLATE 51.

FIG. 7. A peculiar thinning of the hair over the lower portion of the nose.

FIG. 8. The same animal as in Fig. 7. Alopecia areata associated with a slight diffuse infiltration of the skin, desquamation of epithelium, and focal necroses. An unquestionable syphilitic affection.

FIGS. 9 to 13. Affections of the nails.

FIGS. 9 and 10. A bilateral paronychia of the front feet.

FIG. 11. Left hind foot. Marked paronychia and onychia of the nails on the two middle toes and onychia of the inner toe. Hair clipped.

FIG. 12. Right hind foot. Paronychia of first and third toes. Onychia with loss of the nail. Hair clipped on the third toe.

FIG. 13. Simple onychia of hind foot. Hair over the toes clipped. This animal showed a periostitis and necrosis of the terminal phalanges.

PLATE 52.

FIGS. 14 to 19. Cutaneous granulomata analogous in many respects to the grouped nodular or tubercular lesions of the so called secondary and tertiary periods of human syphilis.

FIGS. 14 to 16. Stages in the development of a typical cutaneous granuloma as seen at intervals of 1 week. Fig. 14 shows an area of diffuse thickening in the skin, the surface of which was of a decided copper color. In Fig. 15, there is beginning surface necrosis, and Fig. 16 shows a well developed chancre-like lesion with central necrosis and depressed ulcer. Area shaved.

FIG. 17. A group of six early granulomatous lesions on the dorsum and side of the right hind foot with an older lesion over the tendo achillis as seen after removal of the hair. These lesions were all sharply demarcated but varied in appearance from pale opalescent nodules to nodules of a deep violet-red color. Note the tense appearance of the skin and glistening surface of the two largest lesions.

FIG. 18. A group of granulomatous lesions on the right hind foot representing various stages of development. The lesions in this animal were characterized by an intense violet-red color and by rapid and widespread necrosis. The enlargement of the fifth metatarsal seen in the photograph is due to a syphilitic periostitis. Hair removed.

FIG. 19. Granulomatous lesions of the foot illustrating various stages of necrosis and ulceration and the persistence of the hair over this class of lesion. Hair clipped.

PLATE 53.

FIGS. 20 and 21. A cutaneous granuloma and the scrotal lesions of the same animal. The animal was inoculated in the testicles and subsequently the left testicle was removed, but large scrotal lesions developed on both sides, presenting essentially the same appearance as the generalized lesions of the skin.

FIGS. 22 and 23. An unulcerated granuloma of 4 months duration with a bald patch on its surface and the corresponding lesion of the scrotum 11 months after inoculation. The two lesions were photographed at the same time. A similar lesion in the right scrotum had been excised.

FIG. 24. A subcutaneous granuloma, or gumma, freely movable between the skin and the nasal bones, $26\frac{1}{2}$ months after inoculation. Hair clipped.

FIGS. 25 to 41. Cutaneous infiltrations. The lesions in this group represent processes which are perhaps more analogous to the cutaneous lesions of man than those of the preceding group.

PLATE 54.

FIG. 25. Large copper-colored patches of infiltration just appearing on the right ear. Ears shaved.

FIG. 26. A very early but pronounced area of infiltration on the dorsum of the right front foot. The lesion was of a violet-red color and at this time presented much the same appearance as an early granulomatous lesion. The nodular mass at the carpus is a syphilitic lesion of the ulna. Hair removed.

FIG. 27. Small cutaneous infiltrations at the base of the ears showing accumulation of epithelial scales. An early secondary transformation. Area shaved.

FIG. 28. Multiple lesions of the fore arms and feet grouped in irregular circles with the most active lesions towards the periphery. The affection first appeared in the region of the carpus and subsequently extended as shown in the photograph. The lesions were profusely covered by fine epithelial scales most of which were unavoidably lost in shaving the affected area.

PLATE 55.

FIG. 29. The same animal as in Fig. 28. An annular lesion, the margins of which are partly covered with epithelial scales, and a smaller area of infiltration with a necrotic center covered by a scab. A third lesion is seen in profile upon the anterior surface of the ear. Area shaved.

FIG. 30. The primary lesions of the scrotum in the same animal as that in Figs. 28 and 29, intended to show a similarity in the cutaneous reaction of various parts of the body or between the so called primary and secondary lesions of the skin.

FIG. 31. Multiple cutaneous infiltrations on the right front and hind feet showing some loss of hair and various degrees of necrosis and ulceration. The hair has been clipped but not shaved.

FIG. 32. The scrotum of the same animal and the lesions which developed following inoculation. (See legend of Fig. 30.)

FIG. 33. A large crustaceous lesion over the elbow and paronychia of the fifth toe. Natural appearance.

PLATE 56.

FIG. 34. An early papular lesion on the posterior margin of the ear, showing the pale body of the lesion with a narrow zone of color at its base. Area shaved.

FIG. 35. A small papular lesion of a few days duration on the inner surface of the ear.

FIG. 36. Two small papules with central umbilication surrounded by a network of vessels. Lesions of only a few days duration.

FIG. 37. A group of small papular lesions showing circinate arrangement and bilateral symmetry.

PLATE 57.

FIG. 38. A fleshy papule of the upper eyelid.

FIG. 39. Multiple papular lesions of the upper lid and brow covered by heavy epithelial plaques, a transformation which frequently affects lesions such as that in Fig. 38.

FIG. 40. A fungus type of lesion which appears to be due to a continuous piling up of epithelial debris intermingled with a serous exudate. The body of this lesion was hardly more than 2 mm. in diameter.

FIG. 41. The same lesion at a later date showing a very irregular but still somewhat scaly surface. This figure is given to illustrate modifications which may take place in a given lesion.

FIG. 42. A macular erythema, or roseola, of the ear.

FIG. 43. Cutaneous infiltration following the fading of a roseola. There was still a distinct copper color in these areas.

PLATE 58.

FIGS. 44 to 47. Abnormalities of the skin frequently observed in rabbits infected with *Treponema pallidum* whose connection with the *pallidum* infection is still undetermined.

FIG. 44. An area of alopecia in the region of the loin showing irregular patches of thickening in the skin analogous to those which occur with a new growth of hair. These conditions are especially frequent in infected animals.

FIG. 45. Multiple follicular lesions over the cheek and neck. These lesions develop about the roots of certain hairs, and while they are quite common in infected rabbits, they are occasionally seen in apparently normal animals. Hair clipped.

FIG. 46. Alopecia of the cheek and pale circles in the skin occurring with the healing of lesions such as those in Fig. 45.

FIG. 47. A bare area of skin showing several minute crusts with a bristle protruding and numerous irregular areas of thickening and mottling in the skin. The one marked by the arrow was a definite syphilitic papule. The upper part of the area was shaved about 7 weeks before this photograph was taken but there had been practically no growth of hair within this time, which is most unusual.

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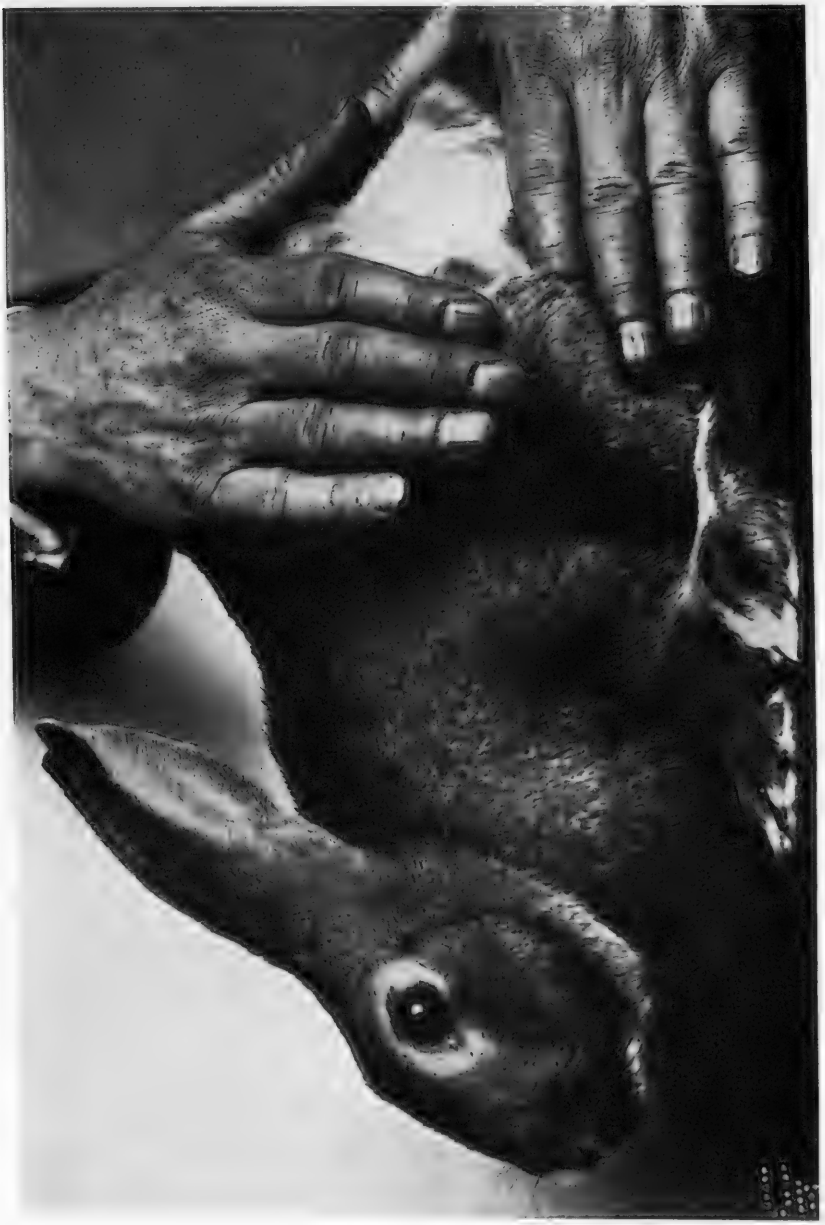
(Brown and Pearce: Experimental syphilis in the rabbit. IV.)

(Brown and Pearce: Experimental syphilis in the rabbit. IV.)



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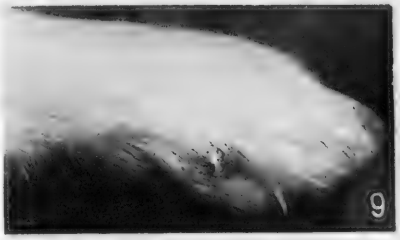

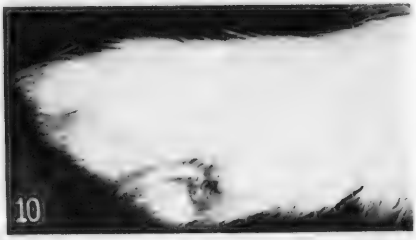

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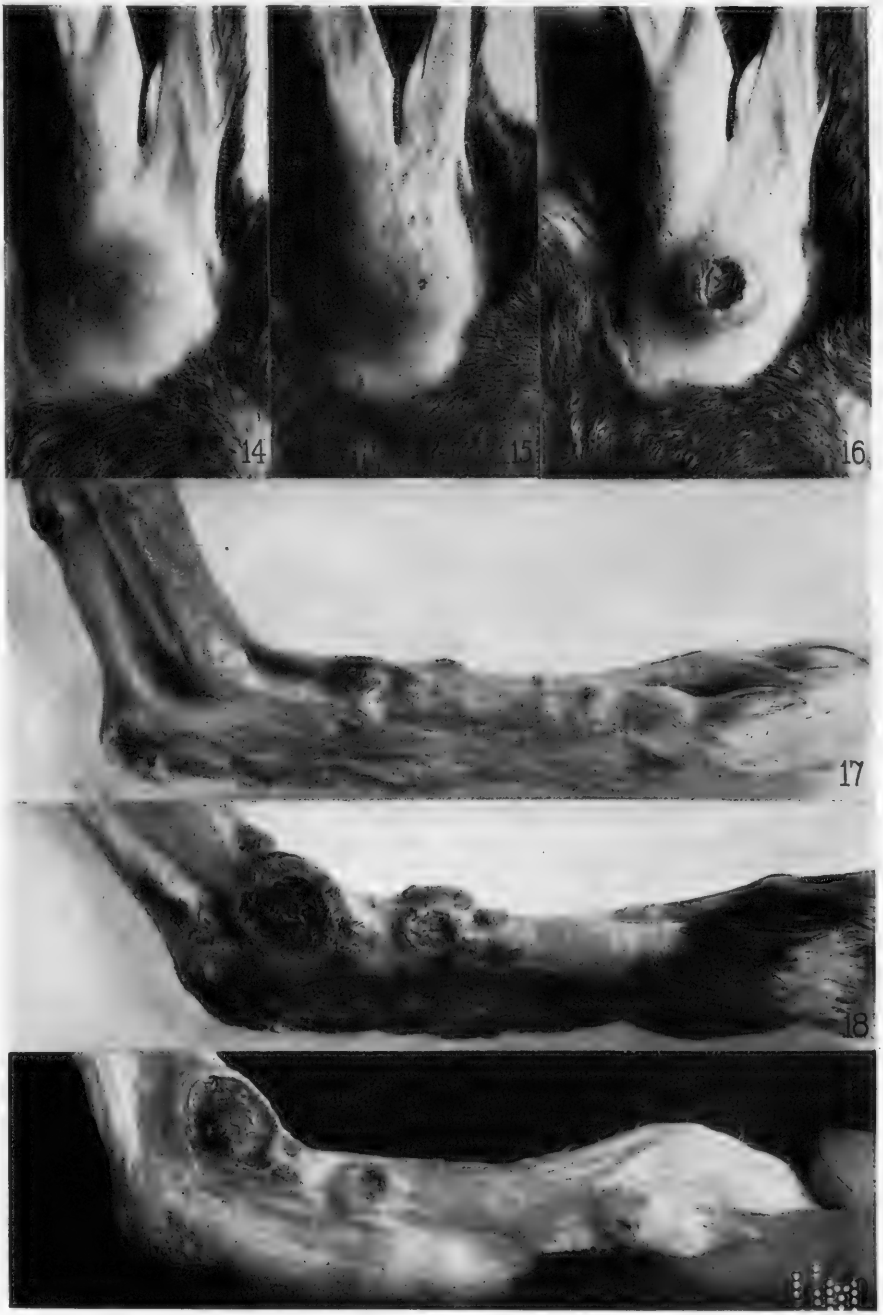

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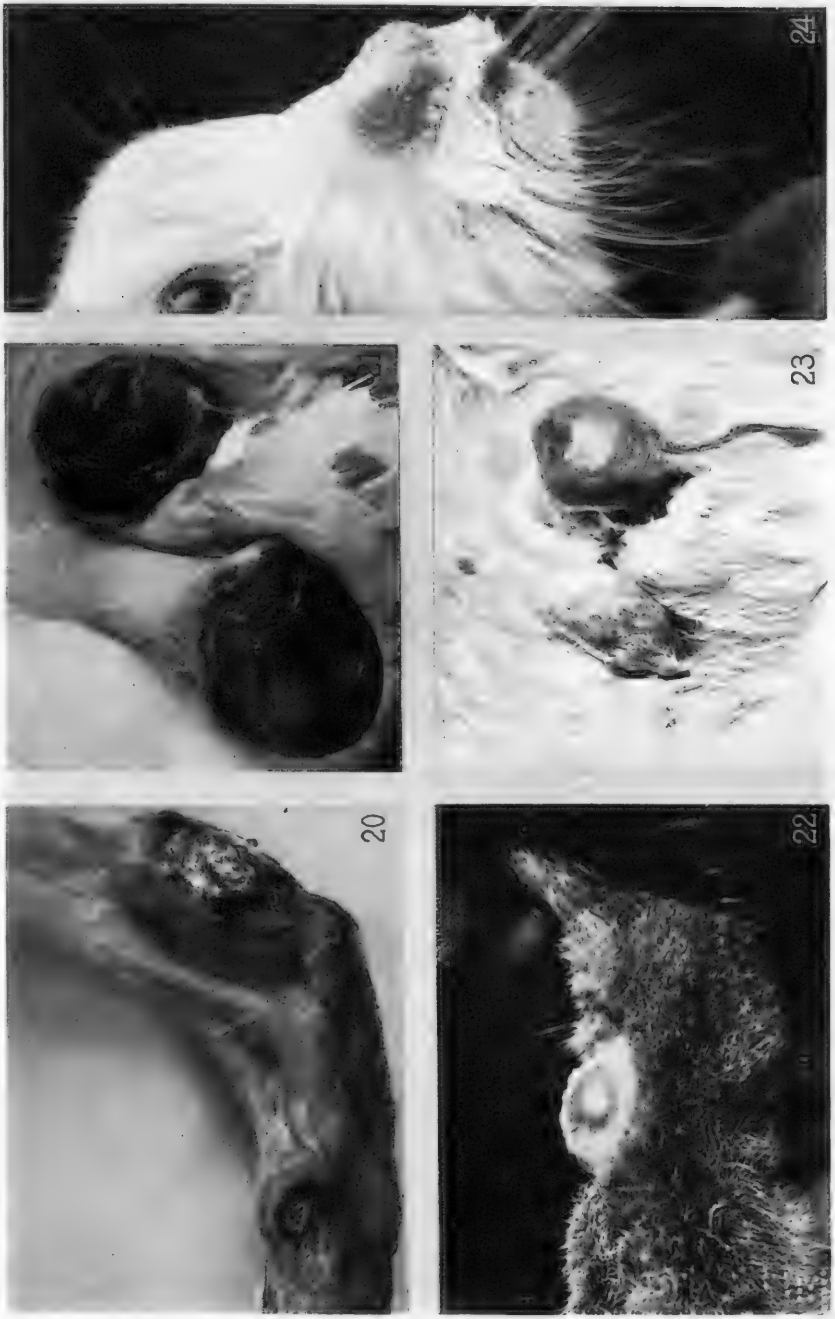

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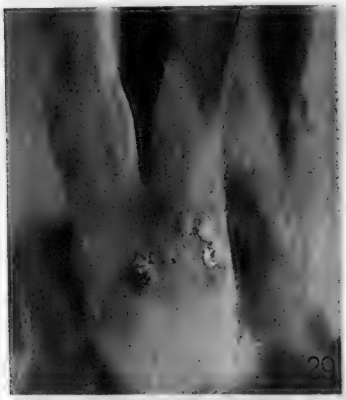

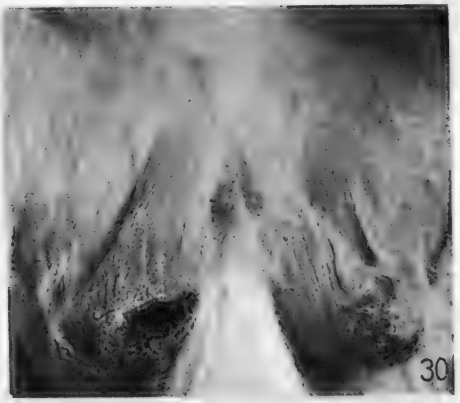

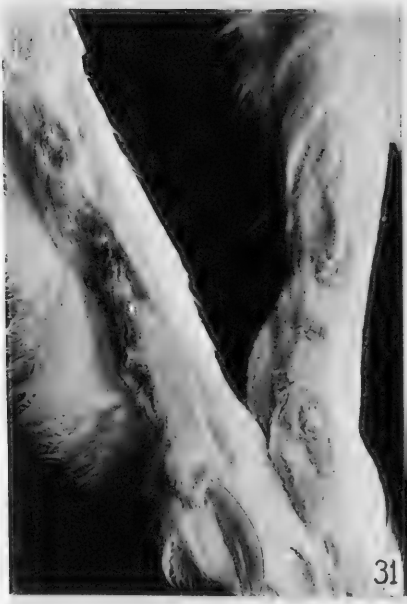

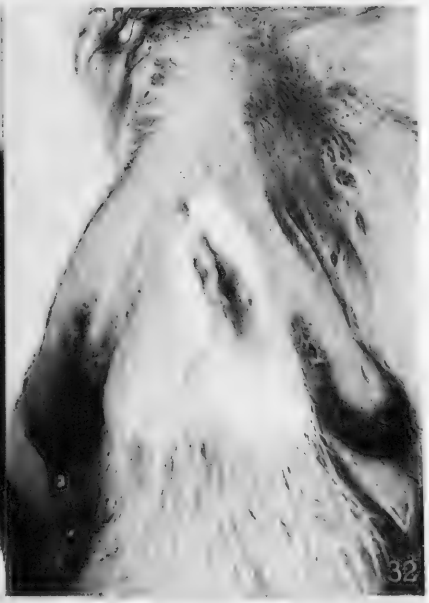

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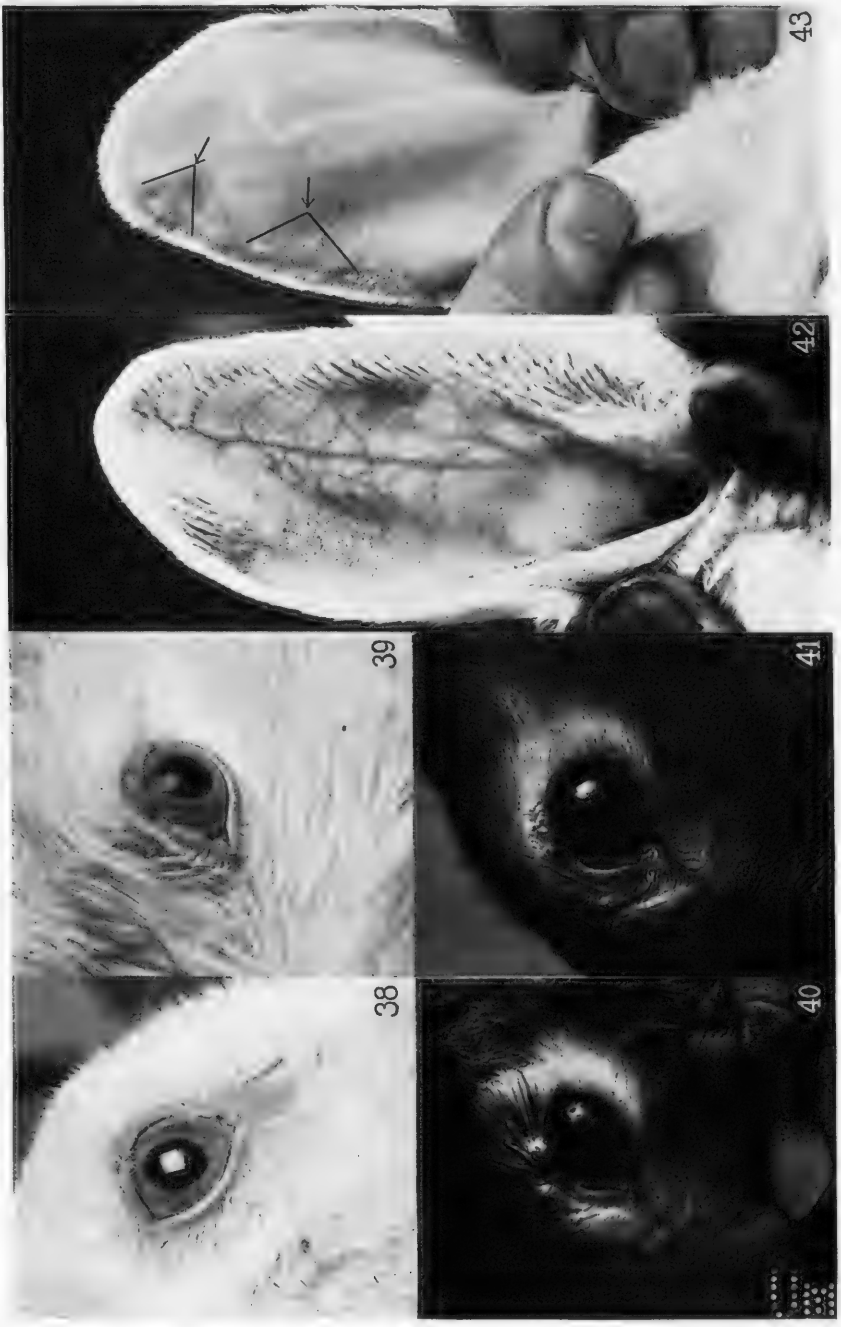

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(Brown and Pearce: Experimental syphilis in the rabbit. IV.)

EXPERIMENTAL SYPHILIS IN THE RABBIT.

IV. Cutaneous Syphilis.

Part 2. Clinical Aspects of Cutaneous Syphilis.

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Plates 59 to 77.

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The character of the cutaneous affections which have been described following local inoculations of *Treponema pallidum* in the rabbit is sufficient to identify these conditions as manifestations of a syphilitic infection and to throw some light upon processes of reaction in the experimental animal. However, the lesions are but the objective signs or the elements of the cutaneous infection, and it will be necessary to give some account of the clinical history of these conditions before a definite place can be assigned to them as integral parts of a generalized infection.

It is the purpose of this paper, therefore, to present such facts concerning the occurrence, distribution, and fate of cutaneous lesions as will enable one to formulate a general conception of cutaneous syphilis in relation to other phases of the experimental infection. In following out this plan of presentation, detailed statements of quantitative relationships will be omitted for the sake of simplicity as well as to avoid any prejudice which might be created from an attempt to assign such values upon the basis of the material which is at present available. Obviously, this aspect of the subject is of importance in itself and will be considered in due time.

Character and Distribution of Cutaneous Lesions in Different Parts of the Body.

From the description of cutaneous lesions which has been given, it is perhaps apparent that these affections tended to preserve a cer-

tain order of distribution and that the character of the lesions present differed somewhat according to their location.

The parts most frequently involved were the hind feet and legs, the head, the front feet and legs, and the tail.[1] This order of distribution was subject, however, to rather wide variations which corresponded in a measure with similar fluctuations in the incidence of different types of cutaneous affections. This is a fact of some importance, since the distribution of the lesions or the character of the lesions present in a given case was undoubtedly influenced to some extent by the particular organism with which the animal was infected. Further than this, the lesions did not occur indiscriminately over those parts of the body which have been mentioned but were confined, for the most part, to a few selected areas.

Lesions of the Head.

Among the animals studied by us, there was a large number which showed involvement of the skin about the head, and the lesions seen in this locality represented practically every form of cutaneous affection which has been described but with a decided predominance of processes of an infiltrative character. The points of especial predilection were certain parts of the face, the brows, the lids, the lips, the base and free portions of the ears, and the cheeks, and the affections peculiar to these areas will be taken up in the order given.

Face.—The lesions which occurred in the skin extending from the nose up over the forehead were chiefly small flattened areas of infiltration or slightly elevated papules, the usual location of which was the sides and bridge of the nose. The appearance presented by animals with affections of this type and the general distribution of the lesions may be illustrated by the photographs reproduced in Figs. 1 to 8. Some of these conditions were so apparent as to be recognized at a glance, but not infrequently there were no visible signs of abnormality and lesions could be detected only by careful palpation of the parts. Thus, the papule on the nose of the animal in Fig. 5 was made conspicuous by its location on a prominent part of the nose and in an area where the hair is short, while a lesion of essentially the same character in Fig. 6 gave no visible sign of its presence.

[1] Lesions about the anus and sheath were not included among the cutaneous affections but with those of mucocutaneous borders, while perineal affections were entirely eliminated for reasons which will be stated below.

As a rule, facial lesions were multiple, bilateral, and symmetrical. The more common ones were of the type represented in Figs. 2, 4, 5, and 6. Occasionally, however, affections of a more pronounced character were seen such as those in Figs. 1, 7, and 8. These were usually single and occupied a position at or near the midline in the region of the bridge or sides of the nose.

Brows.—The brows were less often involved than the face but were the seat of a variety of lesions. Those which appeared to be most characteristic of this area were small indurated papules of the type shown in Figs. 9 and 14, together with focal areas of infiltration and necrosis identical with those of the face. Two such lesions are indistinctly seen in Fig. 4. In addition to these, papules of a larger type or even granulomatous lesions were observed in a few animals, a characteristic example of which is given in Fig. 10.

With two exceptions, the lesions which occurred on the brows were bilateral and were usually accompanied by similar conditions on the upper eyelids (Fig. 14).

Eyelids.—Localized infections of the eyelids, while comparatively common occurrences in the rabbit, gave rise to two groups of conditions which could not be clearly separated from one another. In some instances, the lesions arose from the cutaneous surfaces of the lids and appeared to represent affections of essentially the same status as those of the brows; in others, they were clearly marginal in origin and related to the transitional area, or the infection was confined to the conjunctival surface.

However, the general character of the conditions found upon the external surfaces of the lids may be described without attempting to make any sharp distinction as to their origin.

The simplest form of lesion on both the upper and lower lids was a small papular infiltration of the type shown in Fig. 11, and these were especially common in the marginal areas. Fleshy papules measuring several millimeters in diameter were occasionally seen on the upper lids also (Fig. 13), but the usual affection was a small indurated papule with surface ulceration (Fig. 12) or lesions of a papulosquamous type such as those in Fig. 14. These lesions were often bilateral and multiple and were not infrequently associated with similar affections of the brows.

The distinctive feature of the lesions on the lower lids was their size. As a rule, they were larger and of a more fleshy character than those of the upper lids and exhibited a constant tendency to encroach upon the free margin of the lid. Their surface was usually scaly or covered with small crusts (Figs. 15 and 16), and in two instances, large chancre-like lesions were formed which underwent central necrosis and ulceration as shown in Fig. 17.

Lesions of the lower lid were all unilateral and single.

Lips.—There were comparatively few animals which showed localized infections on the lips, with the exception of those in which the lesions were situated along the margins of the nasolabial folds. The only conditions seen were patches of infiltration analogous to those in Fig. 18 or small indurated papules of the

type shown in Figs. 5 and 6. Other conditions affecting the marginal area will be described elsewhere.

The lower lip and chin were very rarely affected. Thus far, we have seen only three animals with lesions in this location, and all of these were comparatively small papules or areas of infiltration one of which is illustrated in Fig. 19.

Base of the Ears.—The skin at the base of the ears and over adjacent parts of the cheeks and neck formed another area for the localization of cutaneous affections (Figs. 20 to 25 and 83 to 88[2]). The lesions seen here were of two very different types and included processes varying from small papular infiltrations analogous to those in Fig. 20 to larger lesions of a circumscribed or diffuse character, (Figs. 21 to 25). In a few instances, large granulomatous masses such as those in Fig. 23 were seen, but the more common affections were small indurated papules or patches of diffuse infiltrations showing various forms of secondary alteration such as were described and illustrated in Part 1 of this paper. Not infrequently these were associated with similar processes on other parts of the ears or tended to spread from the basal area to adjacent parts of the ears as in Fig. 24.

By reference to the photographs illustrating these conditions, it will be seen that the position of greatest predilection for these affections was the lateral surface of the ears immediately below and at the sides of the intertragal incision. The lesions in this area were usually multiple; in some instances they were confined to one side, but in others they exhibited the most perfect bilateral symmetry (Fig. 23).

Ears.—The auricle was also the seat of cutaneous lesions in a number of animals. In the main, these were of the type of macular or small papular eruptions. The macular lesions have been described in detail and no further comment need be made here.

Upon the outer surface of the ears (Figs. 22 and 24 to 27), comparatively few lesions were seen, and, with the exception of that shown in Fig. 26, they were all situated on the lower portion of the ear and occupied one of four positions: the margins of the intertragal incision (Figs. 24 and 27), the medial surface of the ear (Fig. 25), and the area of the anterior or posterior marginal vessels (Fig. 22).

The majority of these lesions were flattened areas of infiltration covered by silvery or grayish yellow scales with occasional points of necrosis or ulceration covered by crusts; in a few instances they were small indurated papules.

Over the internal surfaces, three types of lesions were noted (Figs. 28 to 31). One of these was the macular erythema (Fig. 31) and another the small papular eruption (Fig. 30) which have already been described. These affections occurred chiefly upon the outer part of the ear and more often near the anterior than the posterior margin. They occurred singly, as in Fig. 28, or in groups, and occasionally exhibited a circinate arrangement or appeared to follow the course of the marginal vessels.

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[2] See Part 1 of this paper also: *J. Exp. Med.*, 1920, xxxii, 445.

A third type of lesion which was seen only once was that shown in Fig. 29. This condition developed as a flattened area of infiltration with slightly elevated margins. The surface was first covered with epithelial scales and later by yellowish gray crusts as shown in the photograph. There was a single lesion of this type on each ear.

Individual lesions on both the internal and external surfaces of the ears varied from a few millimeters to a centimeter or more in diameter and were usually symmetrically placed upon the two ears.

Cheeks and Neck.—With the exception of the area at the base of the ears, there were few lesions seen on the cheeks and neck which could be identified as syphilitic. The small follicular lesions and their various modifications referred to in the preceding paper (Part 1) were the most common conditions observed. In addition, several animals showed a diffuse infiltration of the skin over the cheeks and sides of the neck with a loss of hair and a tendency to desquamation or exfoliation.[3] There was one animal in which the area of involvement extended well down over the shoulder and resulted in the formation of two irregular patches of necrosis.[4] These were the only definite lesions seen in this part of the body. One other animal showed a small flattened lesion of a scaly character in the triangular area at the back of the neck which was probably specific, since at the same time there was a profusion of definite skin lesions elsewhere.

Lesions of the Hind Feet and Legs.

The hind feet and legs of the rabbit hold the distinction of being the most common location of cutaneous lesions (Figs. 32 to 55). This applied especially to granulomatous lesions, and it was here that this type of affection reached its highest state of development. In addition to the granulomata, there were, however, rashes of various types, onychias, and paronychias.

Ordinarily, these affections produced but little change in the general appearance of the parts, and the conditions which one might note were in the main prominent swellings due to the presence of large granulomatous lesions or areas of necrosis and ulceration such as are shown in Figs. 32 to 34. Instances of this kind were comparatively rare, however, and as may be seen by reference to Figs. 35 to 38, which are photographs taken before and after removal of the hair, even marked affections of the skin usually produced only slight changes in appearance.

[3] See the section on alopecia in Part 1 of this paper.

[4] Fig. 8, Part 1.

The lesions on the hind feet and legs were generally located along the lateral margins of the feet more upon the dorsal than the plantar surface, and were distributed from the region of the tendo achillis to the base of the fifth toe. The positions of greatest frequency were the region of the tarsus and external malleolus, the base or tuberosity of the so called fifth metatarsal, the lateral and posterior surfaces of the heel and tendo achillis, and the region of the metatarsophalangeal joint. These peculiarities of distribution and the character of the lesions found in the various locations are brought out by the photographs reproduced in Figs. 36 to 55. It will be noted that the positions enumerated are in a sense pressure points or are skin areas overlying bony or tendinous prominences, and a very large proportion of the cutaneous lesions of the hind feet and legs occurred at these particular points.

Typical examples of the conditions described are illustrated in Figs. 35 to 38 and 39 and 40 which show the hind feet of two animals with marked skin involvement. By reference to the other figures of the series (Figs. 41 to 55), it will be seen that individual variations in the size, number, and location of the lesions were very great. In some instances, frank lesions of the dorsum of the foot or ankle were noted (Figs. 36, 37, and 50), and in their growth those which developed upon the sides of the feet extended in this direction rather than towards the plantar surface. However, plantar lesions or lesions which extended towards the plantar surface were also encountered in some instances (Figs. 51 to 53), and were usually located in the tarsal arch or more exactly in the cushion covering the head of the os calcis just posterior to the base of the fifth metatarsal. Lesions occupying this position are shown in Figs. 49, 51, and 52.

Comparatively few lesions were seen on the legs, and most of these were situated over the tendo achillis, usually not more than a centimeter or so above its attachment to the calcaneus. Occasionally, however, they occupied a much higher position, as in Fig. 46, and in two instances lesions were found on the anterior surface of the shins. One of these is shown in Fig. 36.

The characteristics of distribution described were more closely observed by granulomatous lesions than by those of an infiltrative character. The latter manifested a greater tendency to spread in a linear fashion along the outer side of the foot and might, as in Fig. 54, form an almost continuous line of lesions extending from ankle or heel to the toe, but even here the tendency to localize or to concentrate at certain points was still apparent (Figs. 53 to 55).

As may be gathered from an examination of the accompanying illustrations, lesions of the hind feet and legs were occasionally single

or multiple and unilateral, but in the vast majority of cases they were multiple, bilateral, and symmetrical in their distribution.

After what has been said in regard to similar affections elsewhere, no description of the lesions seems necessary. The illustrations are sufficient in themselves to convey the necessary impressions of the variations in size and character of the several types of lesions to enable one to form a proper conception of the subject.

In addition to the lesions described, mention may be made of the occasional presence of such conditions as onychia and paronychia.

Lesions of the Front Feet and Legs.

The prevailing lesions of the front feet and legs were of essentially the same character as those of the posterior extremities but with a larger proportion of infiltrative processes. The common location of these affections was the extensor and lateral surfaces of the fore arms and the dorsum and sides of the feet as shown in Figs. 56 to 70.

The granulomata were usually situated on the fore arms just above the carpus or on the lateral margins from the level of the carpus to the base of the fifth toe; small multiple lesions were sometimes distributed over the carpus and dorsum of the feet as in Figs. 62 and 65. Occasionally also granulomatous lesions were found at a much higher level on the fore arm (Fig. 64), and in rare instances were located on the flexor instead of the extensor surface (Fig. 59).

The general distribution of the cutaneous infiltrations was much the same as that of the granulomata, but they differed in certain respects. In the case of the infiltrations, the lesions usually appeared along the ulnar margins of the fore arm and carpus instead of the extensor surfaces; they almost invariably extended over the dorsum and sides of the feet (Figs. 66 to 73) and frequently involved the median as well as the lateral surfaces (Figs. 69 and 71). In one instance, large crustaceous lesions were formed about the elbow (Figs. 74 and 75).

The cutaneous granulomata not infrequently appeared as single lesions of a large size or as multiple affections of one fore arm. In contrast to this, the infiltrations were in all cases multiple affections and bilaterally symmetrical in their distribution.

Other conditions affecting the fore paws were onychia and paronychia which were most common on the outer or fifth toe (Figs. 74 and 75), but in several animals other toes as well were involved (Fig. 71).

In connection with lesions of the fore arms, attention may be called to the frequent occurrence of edema during periods of marked activity on the part of

various types of cutaneous lesions, since examples of this condition may be seen by reference to Figs. 59 and 61. In the first of these, a very pronounced swelling about the carpus and foot can be made out, and in Fig. 61, there is a marked edema of the skin and subcutaneous tissues which extends beyond the middle of the fore arm.

In other instances where no edema was present, it was noted that shaving an area where there was syphilitic involvement or the infliction of slight trauma by other means which had no effect upon the skin of a normal animal was frequently sufficient to cause a rapid development of edema which lasted sometimes for days. This urticarial reaction of the skin, if it may be termed such, was not peculiar to cases of cutaneous infection but occurred also in animals in which the lesions were situated in the deeper structures, as in the bones. It suggests, therefore, the action of some toxic influence upon the vascular mechanism of parts adjacent to foci of active syphilitic infection, and this feature of the infection is worthy of consideration in connection with the various cutaneous and other reactions which occur subsequent to the administration of therapeutic agents.

Lesions of the Tail.

Localized infections of the tail form a group of conditions which is somewhat obscure. Uhlenhuth and Mulzer (1) reported "tail tumors" as a frequent occurrence following generalized inoculation of rabbits, and the condition thus described was in most instances a bulbous swelling of the distal end of the tail. Except in this connection, we have seen no mention of tail lesions in the literature. Such conditions do occur, however, and are probably of rather frequent occurrence, but the difficulties surrounding an examination of this part of the body are so great that we were unable to give it the attention which it probably deserved and hesitate, therefore, to commit ourselves as to the frequency and importance of this class of affections.

Again, we have been able to recognize two conditions affecting the skin of the tail (Figs. 76 to 82). The one most commonly observed was a granulomatous lesion generally involving the ventral surface or sides of the tail, rarely the dorsal (Figs. 76 to 81). As may be seen in the accompanying illustrations, these lesions were either single or multiple, and while they occurred at various levels, they were

more often found on the proximal and middle thirds than towards the outer end of the tail.

The second type of affection was that shown in Fig. 82. In these cases, the skin involvement was of an equally marked character, especially towards the end of the tail, but partook more of the nature of a diffuse infiltration. The photograph reproduced in Fig. 82 shows a thinning of the hair and the presence of bald patches over the ventral surface of the tail which characterized this group of affections. Towards the outer end, there is also a small area of necrosis. In one case of this kind, the skin covering the entire tail underwent necrosis and sloughed away.

The six cases of cutaneous syphilis of the tail which have been used for illustration are such as might be recognized without any considerable difficulty, and it will be noted that none of them conforms to the "tail tumor" type of lesion. Bulbous expansions of the end of the tail were observed, however, in a few animals but were always of a relatively slight degree and were difficult to detect because of the fact that the physical alterations present were within the range of normal variations in tail structure. These variations are so great that unless one has an accurate record of the tail of each animal before inoculation or the pathological alterations which take place are quite marked, little can be determined by palpation and the fur must be removed before inspection will be of any material assistance. These procedures we were unable to carry out as a routine part of our work and we are not in a position, therefore, to speak with any degree of assurance as to conditions other than those observed.

Lesions of the Trunk.

With the exception of the perineum, few instances of cutaneous lesions involving the trunk have come under our observation. As previously stated, alopecia was noted in a number of animals, and after removal of the hair from localized areas some peculiar conditions were observed which may or may not have been syphilitic.[2] This is not to be interpreted as evidence that this portion of the body remains uninvolved. On the contrary, it is known that lesions may occur upon the trunk, since a pronounced eruption on the back was one of the conditions described by Grouven (2) in the first case of generalized syphilis reported. It does mean, however, that under

ordinary circumstances, marked involvement such as one frequently observes in other parts of the body is far less common on the trunk. It should be pointed out also that the regions in which minor lesions were most frequently observed were those which were most accessible to examination, and when it is recalled that some of these lesions are extremely inconspicuous, it seems not unlikely that many such affections might escape detection altogether if situated upon the more thickly covered portion of the body. The occurrence of cutaneous lesions of the trunk must be regarded, therefore, as problematical and a field for future investigation.

As regards the perineum, it may be said that lesions in this locality, including those of the genitalia as well as the surrounding skin surfaces, were quite numerous and included two classes of affections, one representing conditions of cutaneous origin and the other affections of the mucocutaneous borders.

The first of these groups comprised lesions which for the most part were closely connected with the seat of inoculation, and we have avoided placing too much stress upon them lest some confusion might arise between extensions from primary lesions and generalization of the infection as it is commonly understood. However, the descriptions of scrotal lesions given in the preceding papers (3–6) will apply equally well to those which arise in this area as a result of generalization of the virus. Other lesions of the perineum will be described in connection with affections of mucocutaneous borders.

Clinical History of Cutaneous Lesions.

An exact statement of the time of occurrence, the duration, and the relative frequency of different types of cutaneous lesions in the rabbit can hardly be given without entering into a detailed analysis of the experimental conditions under which the lesions developed, and must be deferred, therefore, until the subject can be approached from this standpoint. There are, however, certain facts concerning the clinical history of these affections which may be recorded, some of them in greater detail than others.

Cutaneous Eruptions.—The cutaneous eruption in the rabbits usually consisted of only a few lesions occurring singly or in small

groups upon some one part of the body such as the head or the feet and legs. Occasionally, the lesions were more numerous and more widely distributed, several parts of the body being involved at about the same time or in rapid succession to one another.

The order in which the lesions appeared was somewhat irregular. It was not uncommon for several or all of them to make their appearance at about the same time. In other instances, one or two lesions developed, and no others were detected for a week or more when new lesions appeared and were followed by others at rather long and irregular intervals which at times extended over several months, or, after the appearance of the first lesions, others developed at short intervals until the eruption was complete.

How many distinct periods of eruption might occur is impossible to say, since the majority of the animals were not held for any great length of time. Among those which were kept under observation for a year or more, some showed only a single period of cutaneous eruption, while in others, there were several such periods separated by long or short intervals during which no lesions were present or no new lesions appeared.

In some instances, the consecutive eruptions consisted of lesions situated in places not previously involved, while in others, they might be regarded more as relapses than as new eruptions, since the new lesions developed at the site of older ones.

It was usually but not invariably the case that the lesions of the first eruption were of a larger type and more numerous than those of succeeding crops. This applied especially to the granulomata, an instance of which may be seen by comparing Figs. 39 and 40 with Figs. 41 and 42. Figs. 39 and 40 represent the first cutaneous eruption on the hind feet. These lesions healed completely and remained healed for between 4 and 5 months at which time two of them recurred (Figs. 41 and 42).

Another very important feature of the cutaneous eruption was the apparent influence exerted by one lesion or group of lesions upon another. This was best shown in instances where multiple lesions occurred in the same locality, as upon the fore arms and wrists of the animal in Figs. 62 and 63. When, as in this case, a number of lesions made their appearance at about the same time, the growth of most of

them was abortive, and only one or two developed to any considerable extent. It may be recalled that this same condition was pointed out in connection with the development of multiple lesions of the scrotum.

The inhibition thus exerted by one focus of reaction upon another is a phenomenon of the most fundamental importance and furnishes the key to an explanation of many conditions which characterize the experimental infection. In the present instance, it probably accounts for the existence of so few lesions and the unusual size which individual lesions frequently attain.

From this description of cutaneous eruptions, it is apparent that they were exceedingly variable as to the number and character of the lesions present at a given time, the manner of their appearance, and the number of eruptions which might occur in a given case.

Time of Occurrence of Cutaneous Lesions.—Cutaneous lesions were among the earliest manifestations of a generalized infection, but the time of their appearance was subject to very wide variations. This appeared, however, to be partly due to differences in experimental conditions. The first cutaneous eruption usually appeared between the 2nd and 4th months after inoculation and on the whole was earlier in the case of the granulomata than with lesions of an infiltrative character. The earliest recorded time for the appearance of cutaneous lesions was 3 weeks after inoculation, and the longest interval between inoculation and the first cutaneous eruption was 2 years and 8 months; the next longest was between 6 and 7 months. It may be said, therefore, that the first cutaneous eruption appeared at from 6 weeks to 6 months after inoculation with occasional cases occurring earlier or later, depending upon the conditions of the infection.

As regards the occurrence of subsequent eruptions, or the interval between eruptions, very little can be said. In the few animals which were held for any considerable length of time, the period of active eruption rarely extended beyond 4 to 6 months after inoculation. One case has been mentioned, however, in which the first lesions were noted 2 years and 8 months after inoculation. A second eruption occurred in this animal 3 years and 5 months after inoculation, and a third eruption 2 months later. Lesions of this second group are shown in Fig. 52.

Two other animals of our series showed repeated periods of active cutaneous eruption extending over more than 2 years from the time of inoculation, but during this time they were never entirely free from lesions. A third animal now under observation has followed much the same course for a period of 8 months. In a fourth animal, as in the case of the one cited above, there were three sharply separated periods of eruption spaced at intervals of approximately 7 months. These few cases will serve to indicate the difficulty in attempting to set any time limits upon the occurrence of cutaneous lesions when so few animals were kept under observation for any considerable period of time, but they also indicate that cutaneous affections may occur during the late as well as the early stages of the infection.

Clinical Course of Cutaneous Lesions.—The clinical course of the cutaneous infection was essentially the same as that of the testicular or scrotal lesions. The tendency to pursue a periodic or relapsing course was apparent in the development and resolution of individual lesions as well as in the recurrence of healed lesions and the development of successive crops of eruptions.

These features of the skin reaction were again most evident in the granulomata, the growth of which usually proceeded by irregular stages interrupted by periods of inaction or even regression. Relapse of partially or completely healed lesions, which is but an exaggerated form of this reaction, was a comparatively common occurrence and may be illustrated by the series of photographs reproduced in Figs. 83 to 88 representing the state of a lesion at intervals of 90, 97, 105, 133, 141, and 160 days respectively after inoculation. This type of phenomenon was practically constant, but we have little evidence upon which to base an estimate of the frequency of relapse of completely healed lesions. It may be mentioned, however, that of the few animals kept under observation for a year or more, a number showed recurrence, and with three of them there were several periods of complete healing of individual lesions followed by relapse.

Duration of Cutaneous Lesions.—Spontaneous regression and healing were the common fate of all cutaneous lesions, but great differences were found in the duration of different types of lesions as well as of individual lesions. As was mentioned elsewhere, the macular erythemata were characteristically of short duration, sometimes dis-

appearing within 24 to 48 hours and rarely persisting for more than a few days. In like manner, small infiltrations not infrequently disappeared spontaneously within a few weeks after they were first noted.

On the other hand, granulomatous lesions and many of the infiltrations, especially the papular lesions of the brows and lids, were more enduring as a rule. For example, the papular lesions of the brows and lids shown in Fig. 14 lasted more than a year before they completely disappeared, and the lesion at the base of the toe in Fig. 48 is one of a small group which showed an even greater persistence, having lasted for more than 2 years. These were exceptional cases, however, and from the data available, it would appear that the average duration of cutaneous lesions was hardly more than 2 to 4 months, although in a fair percentage of animals, this period might be prolonged by several months.

The duration of active skin infection was somewhat longer than that of the individual lesion, since all the lesions present did not pursue a parallel course of changes. The available data bearing upon this feature of the infection, however, are insufficient to permit of an estimation of the time which such infections might endure.

Detection and Diagnosis of Cutaneous Lesions.

In view of the great frequency with which cutaneous lesions appear to follow local inoculations of *Treponema pallidum* in the rabbit and the fact that so few of these conditions have been described, it seems well to refer briefly to the detection and diagnosis of this class of affections.

The first essential to the detection and diagnosis of cutaneous lesions is obviously a knowledge of the character and distribution of these affections, and this we have attempted to supply. Large granulomatous lesions such as those in Figs. 32 to 34 which produce striking irregularities in the contour of the affected parts, or lesions which have resulted in a considerable destruction of the skin surface, as in Figs. 74 and 75, may be seen almost at a glance if one knows where to look for them. Prominent swellings and surface erosions are not always present, however, and at all events are late phenomena. The great majority of the lesions which affect the feet, legs, and tail are

concealed from view and can be detected only by palpation, rarely by inspection. Lesions about the head are more exposed as a rule, and careful inspection of the regions in which they are known to occur is highly essential, but with the exception of a few areas such as the ears and the eyelids, even here the chief reliance for the early detection of cutaneous lesions must be placed upon palpation.

The technique of this operation consists in picking up the skin and gently rolling it between the thumb and finger. In this way, one is soon capable of detecting the slightest thickening or irregularity in the skin, and if the hair is removed and these initial changes are carefully followed from day to day, little difficulty will be experienced in arriving at a diagnosis.

If the animals are separately caged and well cared for, few conditions will arise which are apt to be confused with typical syphilitic lesions, even the smaller infiltrations and erosions. Apart from diseases of the skin, such as mange, and traumatism inflicted by the animal itself, which are very readily recognized, the only three conditions which need be considered are small focal abscesses, old scars, and focal thickenings due to a new growth of hair. The first two conditions are, in the main, trunk affections and should not be confused with syphilitic lesions. The third condition may occur anywhere and is especially troublesome about the face, but clipping of the hair and careful observation will usually enable one to arrive at a correct diagnosis. Examination for spirochetes may or may not prove helpful in these cases and may even defeat the object of the examination by causing regression of a syphilitic lesion before a characteristic condition has been established.

CONCLUSIONS.

The description of cutaneous syphilis in the rabbit following local inoculation is incomplete in many respects but is sufficient to indicate that a generalized infection of the skin does occur in a large number of animals—just how often and under what circumstances we shall not attempt to say.

It is apparent, however, that the cutaneous affection is a very characteristic one. The lesions themselves bear the marks of a varied

but definite pathological process identical in all respects with the reaction set up in the scrotum by local inoculation of *Treponema pallidum*. The character of the lesions and, withal, their great multiformity, the time relations existing between inoculation and the appearance of the cutaneous lesions, the occurrence of successive crops of eruptions, the relapsing course of the disease, and the preservation of a fixed order of distribution of the lesions, are highly suggestive of cutaneous syphilis in man, and it is believed that as more is known of the experimental infection and with better adaptation of organisms, the analogy will become even closer.

SUMMARY.

From the study of a large number of rabbits with generalized cutaneous syphilis following local inoculation with *Treponema pallidum*, lesions were found most often about the hind feet and legs, the head, the front feet and legs, and the tail. There was further evidence of a selective distribution of cutaneous lesions in the fact that, on a given part of the body, the lesions were usually confined to a few restricted areas. About the head, they occurred almost exclusively on the sides and bridge of the nose, the lids, the brows, the lips, and the base and free portions of the ears. On the front feet and legs, the seat of predilection was the extensor and lateral surfaces of the fore arm, the carpus, and the feet, while on the posterior extremities they were situated upon the dorsum and lateral surfaces of the feet and ankles from the level of the tendo achillis to the base of the fifth toe. The positions of greatest frequency were the region of the tarsus and external malleolus, the base of the fifth metatarsal, the lateral and posterior surfaces of the heel and tendo achillis, and the base of the fifth toe. In many instances, the positions of predilection were exposed positions or areas of skin covering bony or tendinous prominences.

It was also found that the character of the lesions differed somewhat in the various locations. The lesions of the head were mostly small circumscribed papules or processes of diffuse infiltration; on the fore arms and feet, affections of this type were about equally divided with larger granulomatous masses of a chancre-like character, while on

the hind feet and legs, granulomatous lesions were far more numerous than those of any other type and frequently reached a very large size.

The cutaneous eruption usually consisted of only a few lesions confined to some one part of the body, but occasionally they were more numerous and more widely distributed. In this connection, it was noted that when multiple lesions appeared in a given area at about the same time, the growth of most of them was abortive, and, as a rule, only one or two developed to any considerable size. Especial emphasis was placed upon this phenomenon of inhibition as a factor of fundamental importance in the experimental infection.

From clinical observation, it was found that, as a rule, the first cutaneous eruption occurred at from 2 to 4 months after inoculation but might occur either earlier or later, depending upon the circumstances in the individual case. The earliest eruptions appeared 3 weeks after inoculation and the latest 2 years and 8 months, but, as a rule, the time between inoculation and the appearance of the first eruption did not exceed 4 to 6 months.

Successive crops of cutaneous lesions appeared in a number of animals usually within the first 6 months after inoculation. In a few instances, however, there were repeated eruptions extending over a period of 2 years or more, the longest recorded period being 3 years and 7 months.

The duration of individual lesions was found to be extremely variable, ranging from a few days in the case of a macular erythema to more than 2 years in the case of a few granulomatous lesions. The average duration of the lesions appeared to vary somewhat with the nature of the lesion but on the whole was not more than 2 to 4 months. No limits could be fixed, however, for the duration of an active skin infection as a whole.

Again, it was found that the cutaneous infection tended to pursue a periodic or relapsing course. This was seen in the mode of growth and resolution of individual lesions, the occurrence of successive periods of eruption, and the recurrence of completely healed lesions, all of which was interpreted as evidence of the essential relapsing nature of syphilitic infections.

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EXPLANATION OF PLATES.

With the exception of Fig. 31 the illustrations are from unretouched photographs which represent the objects at their natural size. The time given is estimated from the date of inoculation unless otherwise stated.

PLATE 59.

FIGS. 1 to 4. Clinical appearance of animals with syphilitic lesions about the face.

FIG. 1. 58 days. An extensive infiltration of the frontal region showing loss of hair and exfoliation over the affected area.

FIG. 2. 138 days. Multiple foci of infiltration and necrosis distributed over the sides and bridge of the nose. There was a similar condition on the left side of the face.

FIG. 3. 249 days. A diffuse area of infiltration with some loss of hair and desquamation of epithelium involving a portion of the lip and the skin over the side of the nose.

FIG. 4. 188 days. Multiple focal lesions over the lower portion of the nose, the right upper lip, and the brows. Lesions were also present about the nares.

PLATE 60.

FIGS. 5 to 10. Syphilitic lesions of the face and brows.

FIG. 5. 69 days. Early papular lesion on the side of the nose.

FIG. 6. 98 days. Small nodular lesion, side of the nose, which was not visible until the hair was removed.

FIG. 7. 128 days. Circumscribed nodular lesion with central necrosis, situated on the lower portion of the bridge of the nose.

FIG. 8. 169 days. Extension and transformation of the lesion in Fig. 7.

FIG. 9. 138 days. Small papular lesions with apical necrosis on the brow.

FIG. 10. 112 days. Large nodular syphilide with central necrosis and ulceration on right brow.

PLATE 61.

FIGS. 11 to 19. Syphilitic lesions of the lids, brows, and lips.

FIG. 11. 98 days. Early papular lesions on upper and lower lids. There are two small lesions on the margin of the upper lid and a single larger lesion on the lower lid.

FIG. 12. 108 days. Small indurated papule with surface ulceration on the margin of the upper lid.

FIG. 13. 134 days. Large papular syphilide, upper lid, with slight necrosis at the center and considerable desquamation of surface epithelium.

FIG. 14. 13 months. Multiple lesions of the upper lid and brow. These lesions were of the nature of small papular infiltrations covered by heavy epithelial scales.

FIG. 15. 80 days. Large nodular lesion, lower lid. This lesion was of an intense copper color with small patches of scales over its surface.

FIG. 16. 113 days. Nodular lesion of the lower lid similar to that in Fig. 15, showing foci of necrosis covered by crusts.

FIG. 17. 84 days. Large granulomatous lesion of the lower lid. The center is necrotic and covered by a thick crust.

FIG. 18. 65 days. Circumscribed area of infiltration, right upper lip. The surface of the lesion is necrotic and covered by a crust.

FIG. 19. 8 months. Multinodular area of infiltration on the chin. This lesion was of a deep copper color and showed several areas of exfoliation.

PLATE 62.

FIGS. 20 to 23. Syphilitic lesions of the ears, basal region.

FIG. 20. 53 days. Two small areas of infiltration in the skin marked by arrows. These lesions developed no further than the condition here shown.

FIG. 21. 113 days. Multiple nodules in the skin at the base of the ear.

FIG. 22. 101 days. Small circumscribed area of infiltration with central necrosis just below the intertragal incision. A second lesion is seen posterior to this and a third on the anterior surface of the ear. All these lesions showed a central area of ulceration and a marked tendency to accumulation of epithelial scales.

FIG. 23. 128 days. Large granulomatous lesions at the base of the ears showing bilateral symmetry of this class of affection. A few small areas of infiltration are also seen above the nodular lesions on the left ear.

PLATE 63.

FIGS. 24 to 29. Syphilitic lesions on the free portions of the ears.

FIG. 24. 8 months. The same animal as in Fig. 22. Spreading patches of infiltration on the lateral surface of the ear.

FIG. 25. 8 months. The same animal. Syphilitic lesions on the median surface of the ear. One of these was a very small area of infiltration (marked by arrow). The other shows an area of necrosis surrounded by a zone of infiltration.

FIG. 26. 104 days. Indurated nodular lesion on the anterior and outer surface of the ear.

FIG. 27. 13 months. Diffuse area of infiltration with exfoliation and necrosis spreading along the anterior margin of the ear.

FIG. 28. 60 days. Circumscribed nodular lesion on the inner surface of the ear, at an early stage of its development.

FIG. 29. 140 days. Circumscribed area of infiltration with raised edges and necrotic center situated on the anterior margin of the inner surface of the ear.

PLATE 64.

FIG. 30. 97 days. Multiple papular lesions on the inner surface of the ear showing circinate arrangement and bilateral symmetry.

FIG. 31. 132 days. Erythematous patches in the ears showing bilateral symmetry.

PLATE 65.

FIGS. 32 to 34. Clinical appearance presented by animals with large granulomatous lesions of the hind feet.

FIG. 32. 122 days. There are four large granulomatous nodules along the outer side of the right foot; on the left, there is a single large lesion in the region of the metatarsal and a second on the outer side of the heel.

FIG. 33. 122 days. Lateral view of the right foot of the same animal.

FIG. 34. 177 days. The same foot at a later date showing the appearance presented after extensive necrosis and ulceration had taken place.

PLATE 66.

FIGS. 35 to 38. Nodular or granulomatous lesions of the hind feet and legs.

FIG. 35. 69 days. Anteroposterior view before removal of the hair.

FIG. 36. 81 days. Appearance presented at a slightly later date with hair removed. The nodular swellings over the outer portions of the fifth metatarsals are due to periosteal lesions.

FIGS. 37 and 38. Lateral views of the same feet. Note especially the character and distribution of the lesions.

PLATE 67.

FIGS. 39 to 42. Nodular or granulomatous syphilides of the hind feet and legs.

FIGS. 39 and 40. 115 days. Right and left hind feet of the same animal showing the character and location of lesions and bilateral symmetry.

FIGS. 41 and 42. 10 months. Hind feet of the same animal showing recurrent lesions at the base of the fifth metatarsals. Note the relative size of the lesions as compared with the original lesions in Figs. 39 and 40.

PLATE 68.

FIGS. 43 to 46. Granulomatous lesions of the hind feet and legs.

FIGS. 43 and 44. 154 and 148 days respectively. Right and left hind feet of the same animal showing single chancre-like lesions, one over the external malleolus and the other on the lateral and posterior surface of the tendo achillis just above the heel.

FIG. 45. 76 days. Granulomatous lesions over the tendo achillis, side of the heel, and base of the fifth metatarsal.

FIG. 46. 125 days. Large granulomatous lesion situated high up on the lateral and posterior surface of the tendo achillis.

PLATE 69.

FIGS. 47 to 50. Atypical distribution of granulomatous lesions on the hind feet.

FIGS. 47 and 48. 2 years and 2 months. The same animal. Single granulomatous lesions involving the heel of the left foot and the base of the fifth toe on the right. These lesions are the same as those shown in Figs. 32 to 34.

FIGS. 49 and 50. Left and right hind feet of the same animal.

FIG. 49. 91 days. There are four lesions on this foot, one over the external malleolus, a second on the plantar arch, the third on the dorsal surface of the fifth metatarsal, and the fourth at the base of the outer toe.

FIG. 50. 83 days. There are also four clearly defined lesions on this foot, all of which occupy positions on the dorsum of the foot, which is quite unusual.

PLATE 70.

FIGS. 51 to 55. Plantar lesions and lesions of an infiltrative character on the hind feet and legs.

FIG. 51. 75 days. Large granulomatous lesion on the outer and plantar surfaces of the tarsus and a smaller lesion over the tuberosity of the fifth metatarsal. There is a third lesion on the plantar surface at the base of the fifth toe.

FIG. 52. Lesions identical with those in Fig. 51. The point of chief interest is the time of their occurrence which was 3 years and 5 months after inoculation.

FIG. 53. 68 days. Discrete infiltrations over the lateral surface of the foot with a tendency towards a plantar position.

FIGS. 54 and 55. 15 months. Hind feet of the same animal. Multiple infiltrations distributed over the lateral margin of the foot. In Fig. 54, the lesions show necrosis, some exfoliation, and a tendency to fuse with one another.

PLATE 71.

FIGS. 56 and 57. Nodular or granulomatous lesions of the front feet and legs.

FIG. 56. 81 days. Multiple nodular lesions over the extensor and lateral surfaces of the fore arms and feet. Note character and location of the lesions.

FIG. 57. 69 days. The same feet at a slightly earlier period before removal of the hair.

PLATE 72.

FIGS. 58 to 61. Lateral views of the same animal as that in Figs. 56 and 57. Note the presence of a lesion on the flexor surface of the fore arm and the edematous swelling shown about the carpus and foot in Fig. 59 and extending to the middle of the fore arm in Fig. 61.

PLATE 73.

FIGS. 62 to 64. Nodular and granulomatous lesions of the fore arms and feet.

FIG. 62. 113 days. Early multinodular affection with lesions on the extensor surface of the fore arms, carpus, and dorsum of the feet.

FIG. 63. 128 days. Later stage in the development of the same lesions. Note the disappearance of most of the lesions shown in Fig. 62 and marked development of single lesions on each fore arm.

FIG. 64. 84 days. Large chancre-like lesion on the extensor surface of the fore arm situated at an unusually high level.

FIG. 65. 60 days. Early nodular syphilides, dorsum and lateral surface of the foot. The prominent swelling over the carpus was due to a periosteal lesion.

PLATE 74.

FIGS. 66 to 70. Infiltrative lesions on the fore arms and feet.

FIGS. 66 and 67. 202 days. Right and left front feet and legs of the same animal. The lesions here shown were in the beginning parchment-like areas of infiltration (Fig. 66) which underwent necrosis and ulceration (Fig. 67).

FIG. 68. 138 days. Appearance presented by an early lesion similar to those in Figs. 66 and 67. The presence of a lesion is suggested by partial loss of hair and discoloration of the skin shown on the lateral surface of the carpus.

FIG. 69. 157 days. Diffuse infiltration on fore arm, carpus, and dorsum of the feet. Hair clipped.

FIG. 70. 184 days. Lateral view of the lesion in Fig. 69, taken during a period of marked activity.

PLATE 75.

FIGS. 71 to 75. Cutaneous infiltrations, front feet and legs.

FIG. 71. 15 months. Frontal view of the feet to show lesions present over the median surface of the toes.

FIGS. 72 and 73. The same as Fig. 71, showing lesions on the lateral surface of the fore arm and at the base of the toes.

FIGS. 74 and 75. 8 months. There is an area of widespread infiltration over the elbows with surface necrosis, exfoliation, and the formation of weeping patches; there is also a well marked paronychia of the fifth toe on both sides.

PLATE 76.

FIGS. 76 to 82. Syphilitic lesions of the tail.

FIG. 76. 77 days. Early multiple granulomatous lesions on the ventral and lateral surface of the tail.

FIG. 77. 76 days. A large granulomatous lesion near the base of the tail with diffuse infiltration of the skin over the outer portion indicated by the glistening character of the surface.

FIG. 78. 65 days. Multiple lesions of a nodular character on the distal portion of the tail. There are beginning necrosis and ulceration of the largest of the lesions.

FIG. 79. 76 days. The same animal, showing extension of the tail involvement together with necrosis and ulceration of the affected area.

FIG. 80. 113 days. Circumscribed nodular lesions, middle and outer third of the tail. The scar of a former lesion is seen in the area marked with an arrow.

FIG. 81. 68 days. A group of three nodular lesions situated on the ventral and lateral surfaces near the base of the tail.

FIG. 82. 109 days. A diffuse infiltrative process involving the skin of the tail over its entire extent. Note the alopecia over the ventral surface of the tail and a small area of necrosis and ulceration near its distal extremity.

PLATE 77.

FIGS. 83 to 88. Periods in the history of a cutaneous lesion.

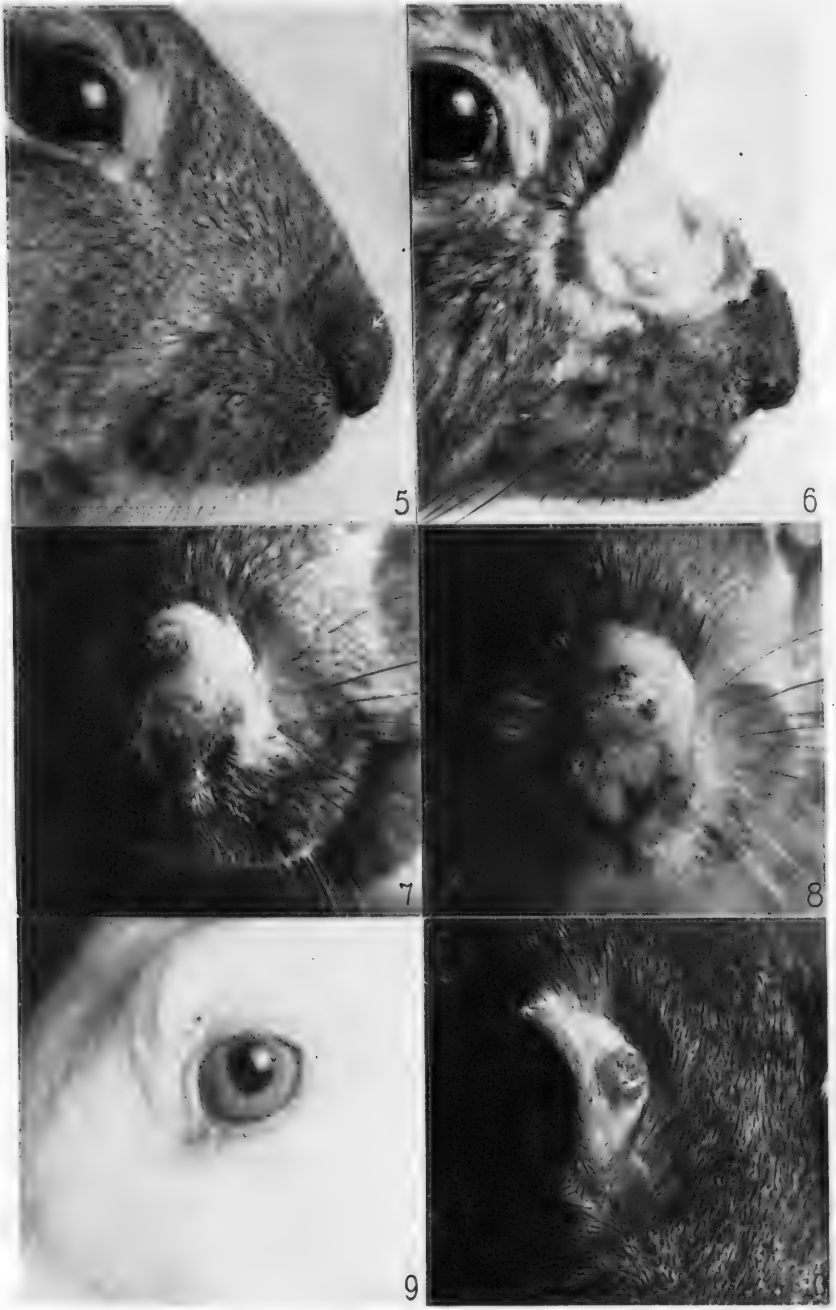
FIGS. 83 to 85. Stages of active development of the lesion. 90, 97, and 105 days.

FIG. 86. 133 days. Marked regression of the lesion.

FIG. 87. 141 days. Definite renewal of activity.

FIG. 88. 160 days. A further stage in the growth of the lesion.

(Brown and Pearce: Experimental syphilis in the rabbit. IV.)



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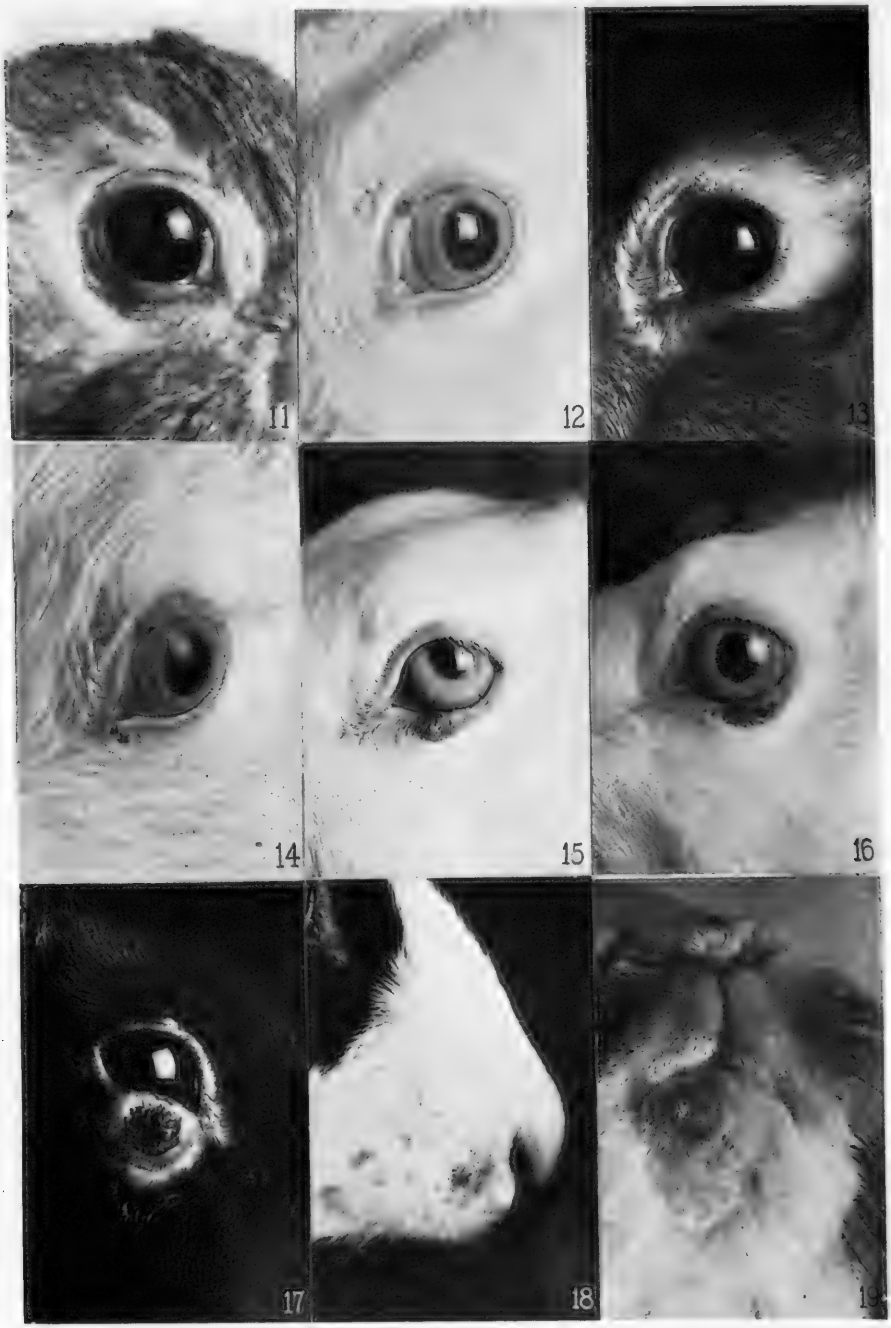

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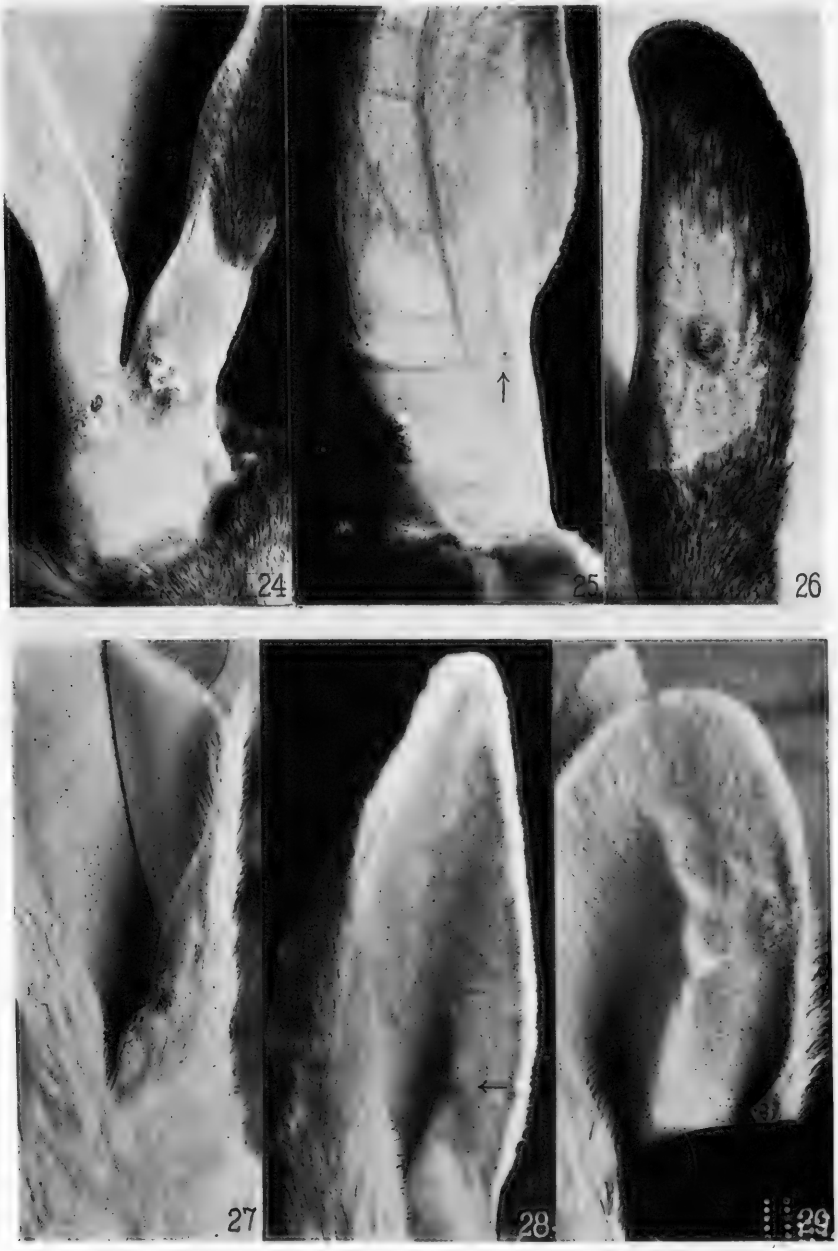

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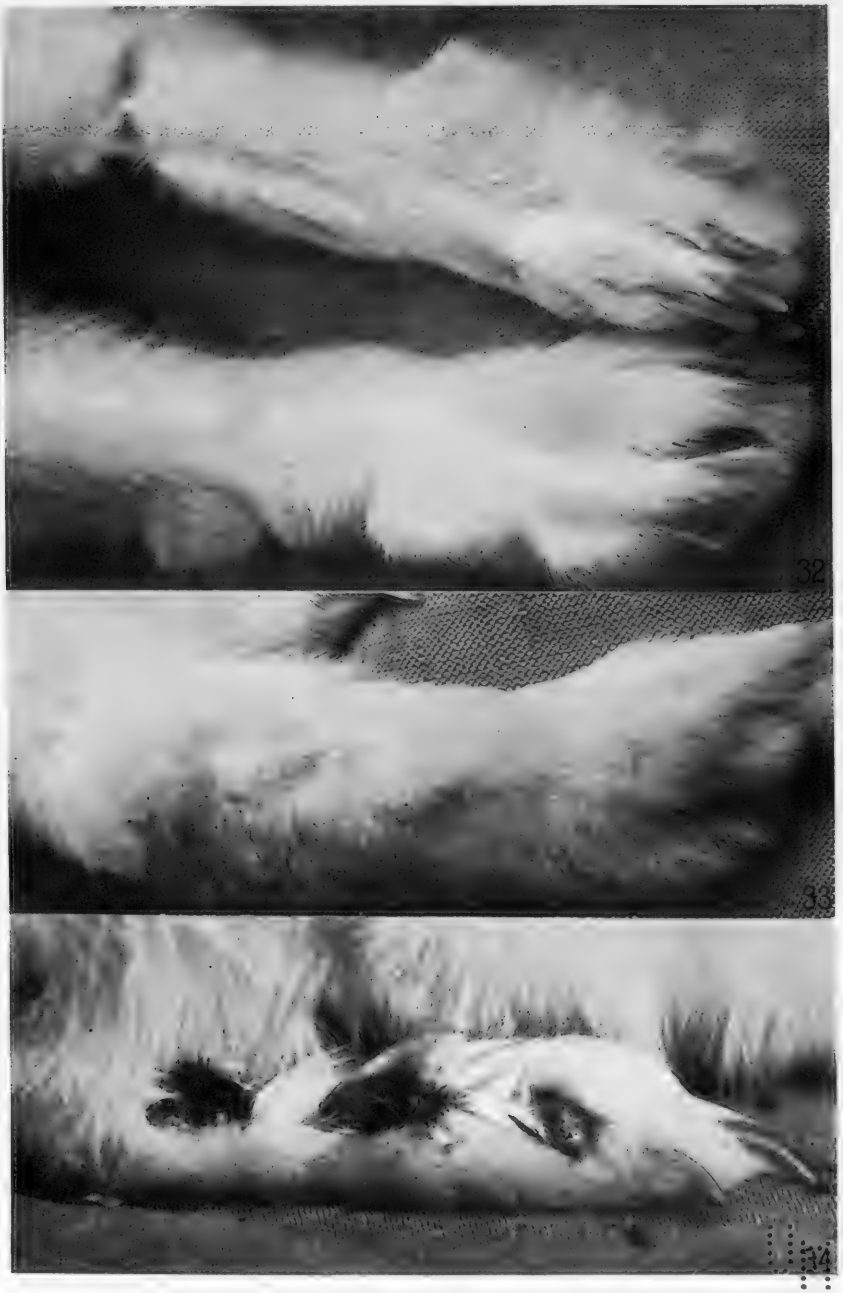

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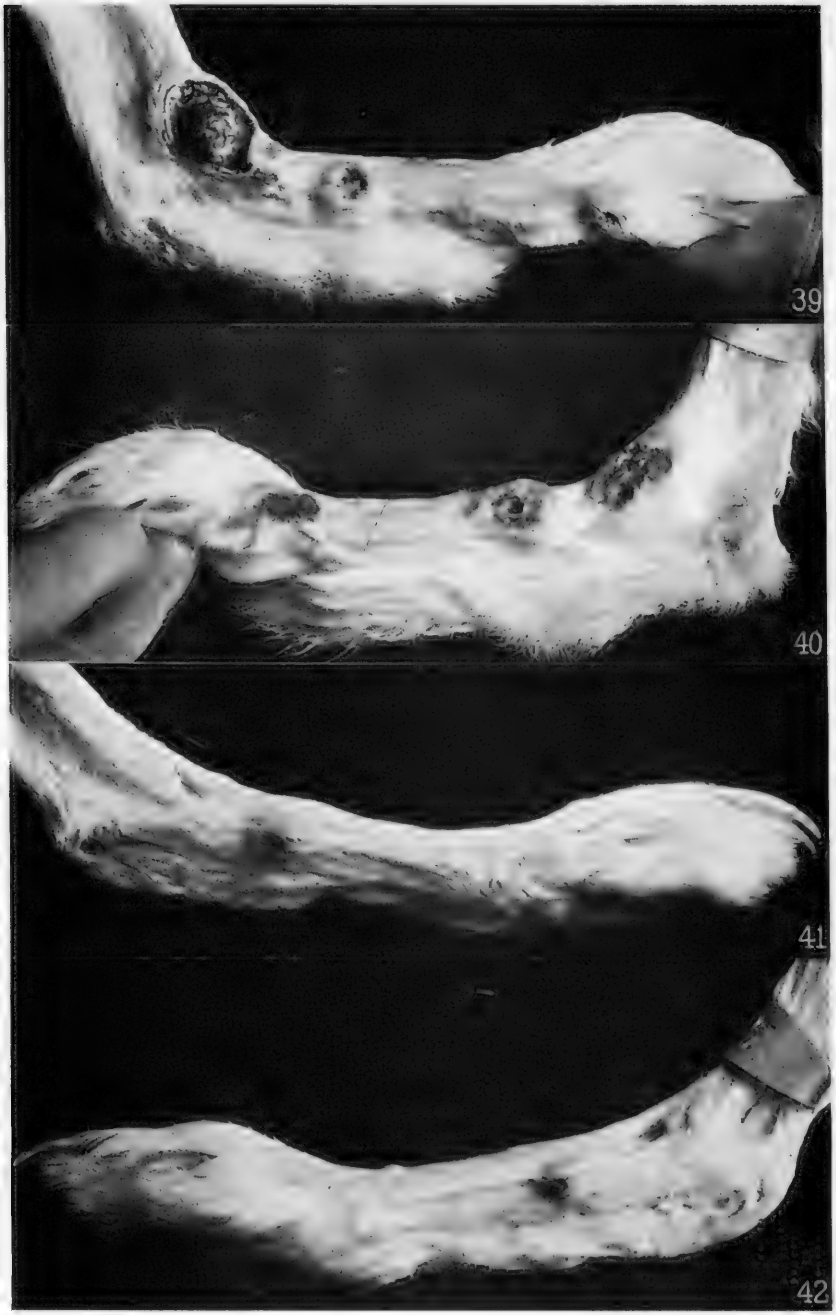

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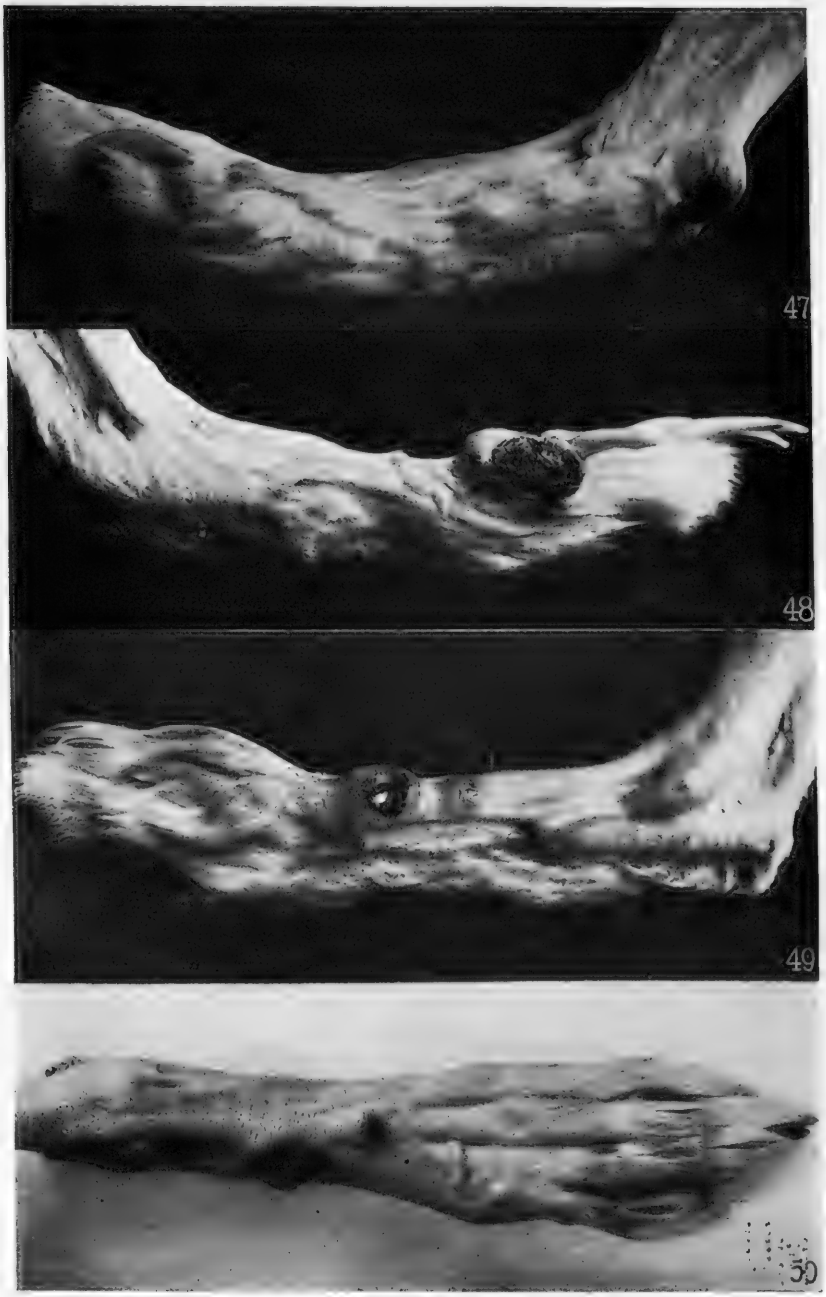

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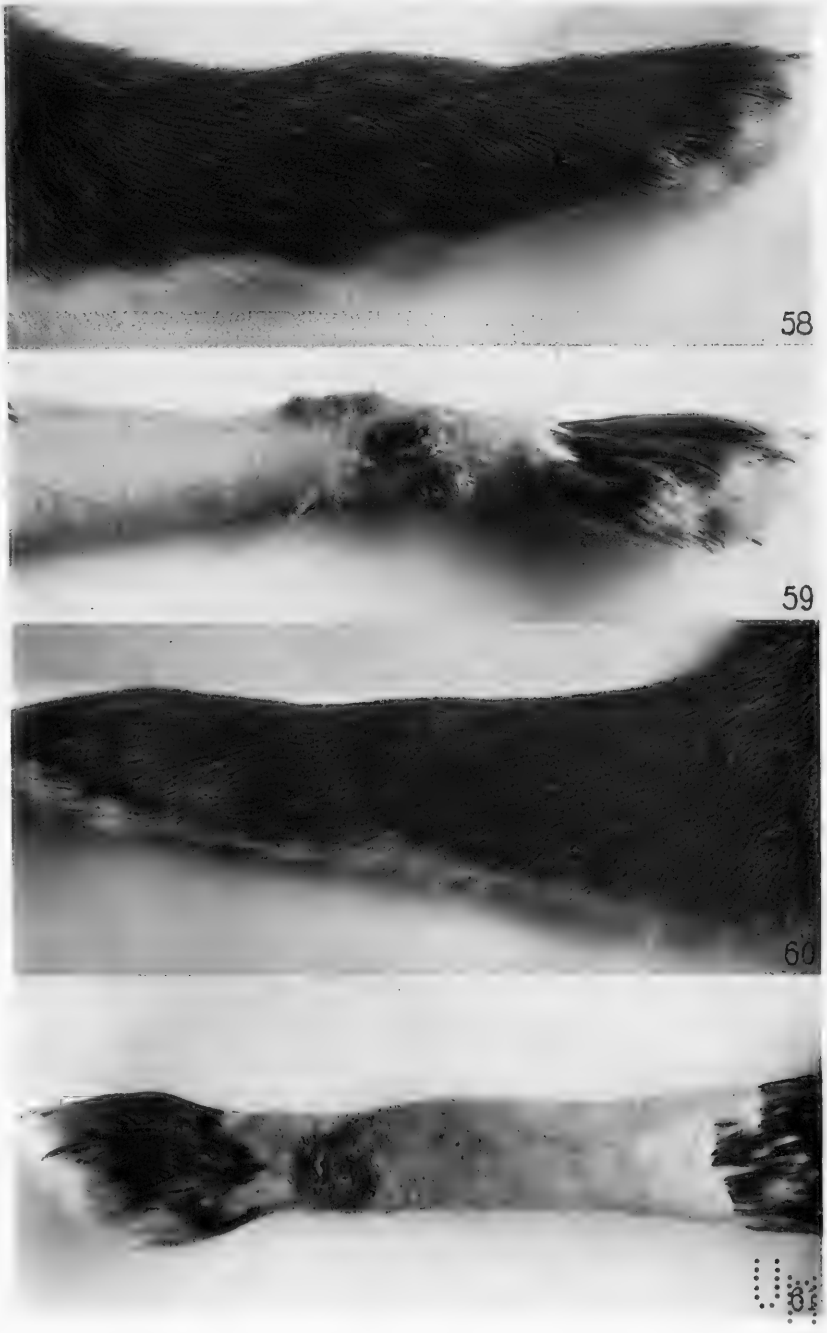

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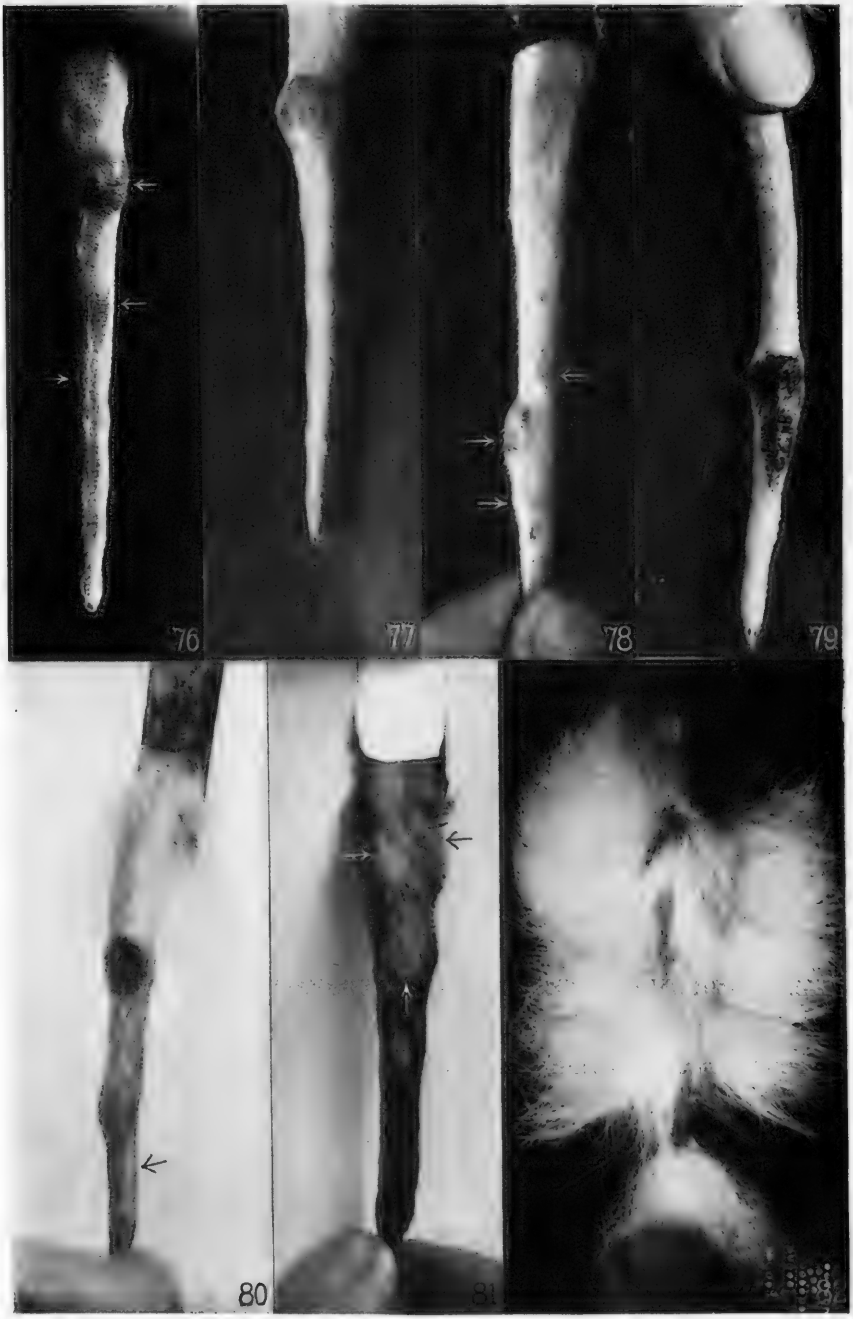

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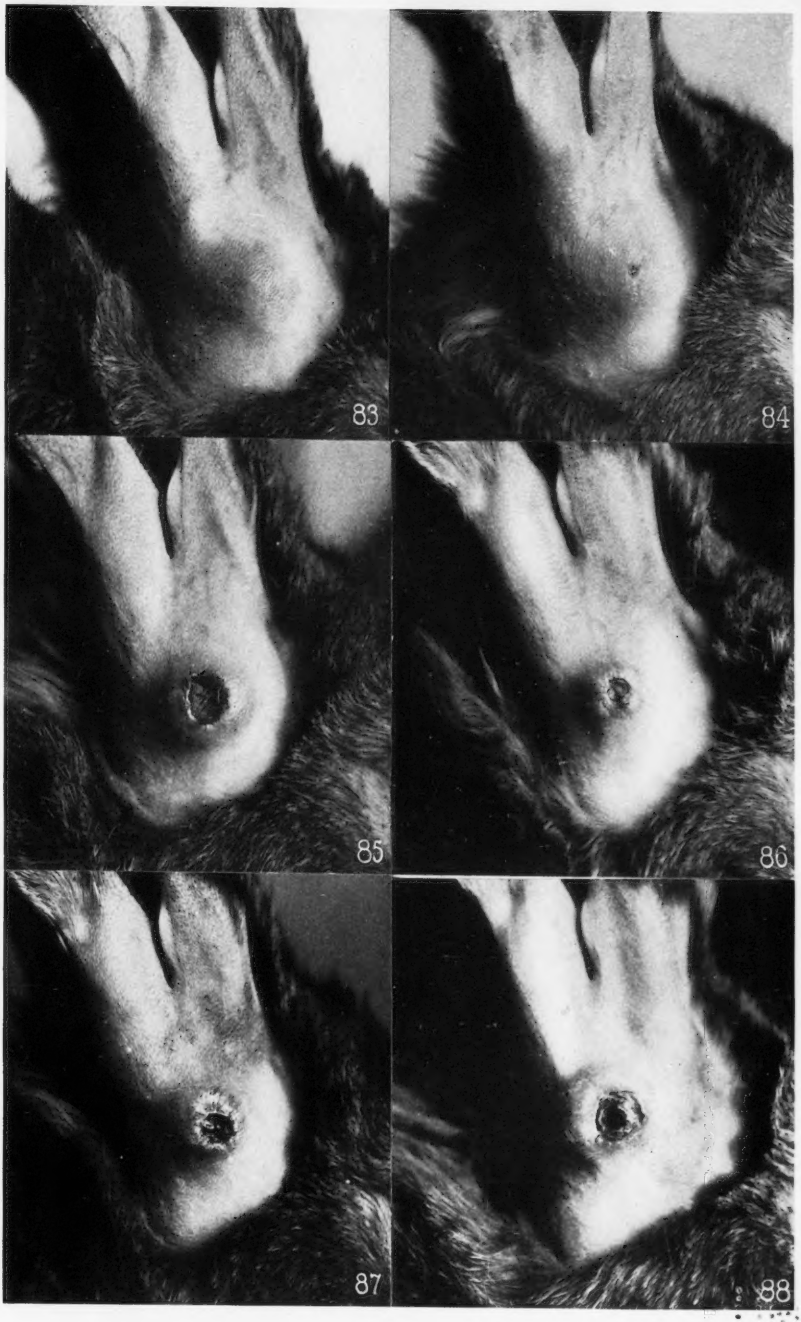

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